UNIVERSITY OF ALBERTA

BARLEY β-GLUCAN: EXTRACTION, FUNCTIONAL PROPERTIES AND INTERACTIONS WITH FOOD COMPONENTS

BY

ZVONKO BURKUS

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

IN

FOOD SCIENCE AND TECHNOLOGY

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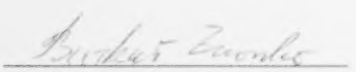

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ABSTRACT

The effect of extraction conditions on yield, purity, viscosity and stability of barley β-glucan (BBG), its interactions with food components and gelation behavior were studied.

BBG was extracted at pH 8-10 and 55°C with four treatments: high pH (P), boiling of extract (B), refluxing of flour (R) and Termamyl treatment of extract from refluxed flour (RT).

B and RT gums were viscous (<100 cP) and stable. Maximal yield (5.4%), purity (75.3%) and viscosity (48.8 cP, 0.5% soln) were achieved with RT gum at pH 8. BBG exhibited synergy with milk, whey and Na-Caseinate gaining 26-250% in viscosity of 0.5-1% solutions, but caused casein precipitation. Interaction with starch was of additive type with instability over time. Interaction with salts was negligible at salt concentrations ≤0.5%. Low viscosity BBG gum formed a gel at 5% level. Gel firmness increased upto 3.5-fold within the concentration range 5-6%. BBG gel was firmer than cornstarch or pure β-glucan gel. BBG shows potential as a thickener and fat replacer in a variety of food products.

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1. LITERATURE REVIEW

1.1. BARLEY PRODUCTION, CONSUMPTION AND VARIETIES

Barley competes with wheat for the throne of the most ancient cereal crop. It was grown along the Nile river as early as 18,300 years ago (Wendorf et al., 1979). Since then, barley has spread out all over the world and today it is the most widely grown crop. Due to its cold, drought, alkali and salt tolerance, it is grown at 70°N latitude in Norway, as well as in regions close to the equator at high altitudes (Poehlman, 1985). Even in some tropical and subtropical areas it is grown during the winter to fulfill the agricultural cycle (MacGregor and Fincher, 1993).

1.1.1. Production

Today barley ranks fourth after wheat, rice and corn in the world cereal production. It is grown on about 78.5 million ha yielding about 175 million t or 2.2 t/ha (U.S. Feed Grains Council, 1990). Average yield can vary substantially from 0.3 t/ha to as high as 10 t/ha under high input conditions in some European countries.

In Canada, barley production ranks second after wheat. It is grown mainly in the prairie provinces of Alberta, Saskatchewan and Manitoba. During the 1980's barley occupied 4.7 million ha of land yielding about 12.6 million t or 2.7 t/ha (Statistics Canada, 1989). Of this total amount, Canada used 90% (8.4 million t) for feed and 10% for brewing purposes. The most recent data (Statistics Canada, 1995) show that Canada produced 13,034,700 t of barley from 4,365,000 ha with

an average yield of 2,986 kg/ha in 1995. Alberta is the leading province in barley production. In 1995, Alberta farmers harvested 6.336 million t from 1.902 million ha with the highest average yield reaching 3,331 kg/ha (Statistics Canada, 1995).

1.1.2. Human consumption

Human consumption of barley is almost negligible mainly due to its less than desirable baking characteristics. Barley does not contain gluten proteins which are necessary for the structure of bread. In addition, barley flour binds more water than wheat flour resulting in a heavy and sticky barley bread. However, barley flour and cracked barley have been successfully incorporated into bread formulations replacing wheat flour up to a level of 50% (Hawrysh et al., 1996). By binding more water, barley flour improves baking economics. Moreover, it can be used for making pastas, flat breads and an assortment of similar products where volume is not of primary concern.

Use of barley flour also improves the nutritional value of such products. Barley contains more lysine, the limiting amino-acid in cereals, than wheat (3.5% versus 2.9%) (Chung and Pomeranz, 1985). Considering the availability of new high-lysine varieties of barley, such nutritional improvements can potentially be quite substantial. The high fiber content of barley, 11-21% in hulless varieties (Newman and Newman, 1991), can add significantly to the quality of the diet of an average North American where it is recommended to consume 20-35 g fiber/day (American Diabetes Association, 1994) .

1.1.3. Barley varieties

The vast majority of barley grown both worldwide and in Canada for the malting and feed industries are hulled varieties of two-rowed and six-rowed type, respectively. If hulled barley is used for human food, it is usually the two-rowed type since it has plump kernels and it is easier to process by abrasion into pot and pearl barley. During such processing, the most valuable parts of the kernel, germ, aleurone layer, fibrous pericarp and β-glucan rich subaleurone layers are lost in the feed fraction while the starchy endosperm, rich in the lower valued hordeins, is retained as the main product. The chemical composition of barley is presented in Table 1.1.

The introduction of new hulless varieties of barley opened a new niche for value added products in the food industry. Transportation costs are reduced since the hull is removed during threshing. The whole kernel can be processed similar to wheat. However, barley bran cracks easily and can not be separated as efficiently as wheat bran (Bhatty, 1992a). This property results in a higher fiber content in the flour. If certain milling procedures are applied, it is possible to obtain barley flour which is comparable in whiteness to wheat flour (Bhatty, 1986). Other properties like water binding capacity may play an important role in food applications of hulless barley. Recently, hulless varieties, which had been grown to a lesser extent, have begun to gain importance in Western Canada. The areas seeded by two-rowed and six-rowed hulless cultivars, 86,480 ha in total, are shown in Table 1.2.

Table 1.1. Chemical composition of barley grain[1].

Component	Dry weight (%)
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water soluble polysaccharides	1-1.5
Alkali soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2-3
Protein	10-12
Albumins and globulins	3.5
Hordeins	3-4
Glutelins	3-4
Nucleic acids	0.2-0.3
Minerals	2
Others	5-6

[1]From MacGregor and Fincher (1993).

Table 1.2. Area (ha) seeded with hulless barley cultivars in Alberta and Western Canada in 1995/96 [1].

Region	two-row barley	six-row barley
Alberta	16,621	10,606
Western Canada	51,348	35,132

[1] Gosain (1996b).

Recent advances in barley breeding have led to the development of waxy hulless barley varieties with special traits for food applications. The area seeded with waxy types is negligible and is not of great importance at present (Gosain, 1996b). Waxy varieties have less amylose than other varieties. They contain ≥95% amylopectin in starch (MacGregor and Fincher, 1993). Another property of this barley type is its increased total dietary fiber (TDF) and soluble dietary fiber (SDF) content. The fiber component which attracted increasing attention is (1→3)(1→4)-β-D-glucan which will be referred to as β-glucan in this thesis.

1.2. β-GLUCAN

1.2.1. Health effects of β-glucan

β-Glucan was reported to have numerous health benefits. β-Glucan lowered blood cholesterol in laboratory animals such as rats, chickens and hamsters (Newman et al., 1992; Kahlon et al., 1993) as well as in humans (Pick, 1994). The ratio between high density lipoproteins (HDL) which is considered to be the "good cholesterol" and low density lipoproteins (LDL) or "bad cholesterol" was changed in favor of the former (Pick, 1994). The exact mechanism of this change is still uncertain. Some researchers are in favor of trapping effects toward cholesterol and its metabolites in a viscous slurry in the digestive tract preventing their absorption (Ilman and Topping, 1985), while others support the beneficial effects of short chain fatty acids produced by colonic bacterial fermentation of β-glucan (Chen et al., 1984). Short chain fatty acids stimulate the immune system and exert an antitumor effect with regard to colon cancer (Fastwood, 1987). β-

Glucan may be useful in controlling blood glucose levels. By increasing food viscosity they slow down glucose absorption and decrease the need for insulin in diabetics (Anderson, 1980; Pick, 1994; Gosain, 1996a).

In contrast to the above benefits, β-glucan can have some negative effects on health mainly due to high β-glucan viscosity and emulsifying properties. Higher viscosity of the intestinal contents slows down the absorption of nutrients in humans and leads to a decrease in weight gain especially in young animals (National Research Council, 1979).

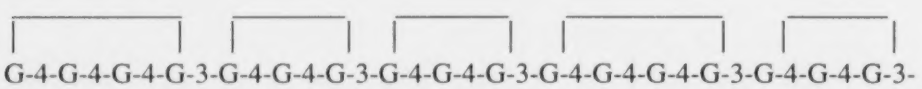
1.2.2. Occurrence and levels

The richest source of (1→3)(1→4)-β-D-glucan are cereals and lichens (Wood, 1984). In cereals, β-glucan is a component of the cell wall structure as well as a reserve polysaccharide. Among the cereal grains, barley and oats have the highest β-glucan content even though levels can vary substantially. β-glucan is present in wheat at a level <1% (Beresford and Stone, 1983) while in barley it can range between 2-11% (w/w), but more usually 4-7% (MacGregor and Fincher, 1993). Oats contain 4.8-6.6% β-glucan (Prentice et al., 1980), but it can be as low as 2.5% (Anderson et al., 1978). Hulless barley varieties are richer in β-glucan than oats. In oats, β-glucan is more concentrated in the outer layers. Thus, after roller milling, oat bran contains most of the β-glucan. The distribution of β-glucan in the barley kernel is similar to that in oats, but the difference between the outer layers and the endosperm is not so sharp (Miller and Fulcher, 1994).

Consequently, a substantial amount of β-glucan is left in the starchy endosperm after pearling (Temelli and Burkus, 1995).

1.2.3. Structure of (1→3)(1→4)-β-D-glucan

(1→3)(1→4)-β-D-Glucan is a polymer of glucose somewhat similar to cellulose. While cellulose contains only (1→4)-β-linkages which tend to form crystalline regions, β-glucan has short (1→4) linked chains made of 2 - 3 glucose units which are connected with (1→3) bonds. If the (1→4) chains are longer, β-glucan tends to behave like cellulose and may even precipitate out of solution if these chains are longer than 6 - 7 units (Table 1.3). Simply shown in the diagram below is the structure of (1→3)(1→4)-β-glucan where numbers 3 and 4 represent (1→3) and (1→4) linkages, respectively, and brackets represent cellotriosyl and cellotetraosyl units (MacGregor and Fincher, 1993).



There is no uniform distibution of cellotriosyl and cellotetraosyl units in the macromolecule of β-glucan. Such a structure makes up about 90% of barley β-glucan (BBG). The detailed structure of β-glucan is shown in Figure 1.1 (Wood, 1984). Data for its molecular weight vary substantially ranging from $2x10^4$ to $4x10^7$ depending on the method used for its determination (Wood, 1984). Woodward et al. (1983) proposed polymerization of up to 1400 units of glucose in the fraction extracted with water from ethanol inactivated barley flour at 40°C and precipitated with the addition of 30% ammonium sulfate.

Table 1.3. Composition and structure of water soluble (1→3)(1→4)-β-glucans from barley flour[1].

Property	40°C soluble fraction	65°C soluble fraction
Monosaccharide composition (% w/w)		
Glucose	98.3	100
Arabinose	1.1	0
Xylose	0.6	0
Linkage positions (% mol/mol)		
(1→3)-β-glucosyl	28	31
(1→4)-β-glucosyl	72	69
Protein (%N x 6.25)	1.2	2.6
Uronic acid (% w/w)	0.1	3.3
Length of block of adjacent (1→4) linkages (% w/w)		
1	4	4
2	56	60
3	32	30
4	5	5
5	1	1
6-7	<1	0
9 (precipitated material)[2]	7.4	7

[1] From MacGregor and Fincher (1993).

[2] Material precipitated during hydrolysis and represents a range of 6-14 adjacent (1→4) linkages. The values are not included in calculation of lengths of blocks that remain in solution after hydrolysis of glucan by (1→3)(1→4)-β-glucanase.

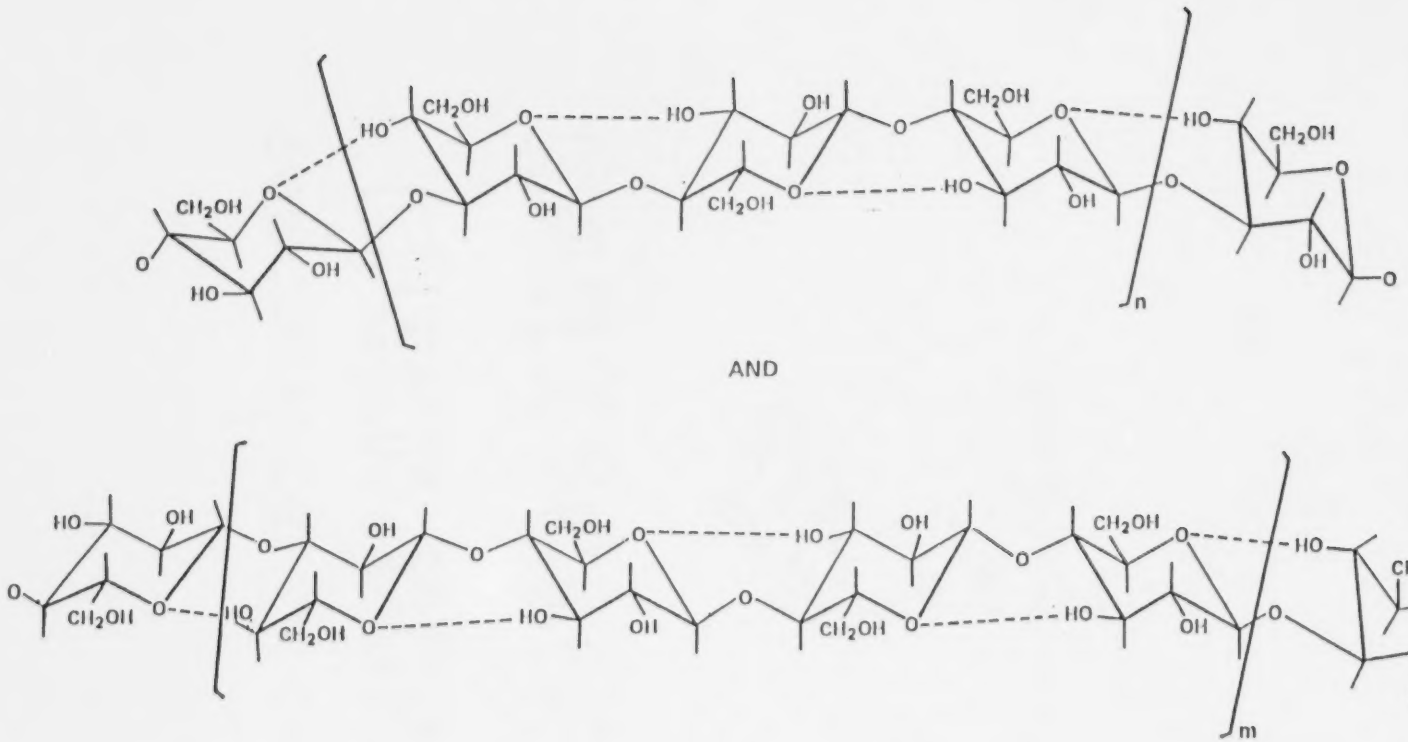


Figure 1.1. Structure of cereal β-glucan.

1.3. EXTRACTION OF β-GLUCAN GUM

β-Glucan gum is a concentrate of (1→3)(1→4)-β-D-glucan obtained from oats or barley by means of solvent extraction, precipitation with alcohol and drying. Its β-glucan content usually varies between 65 to 85%, with proteins, starch, ash and pentosans being the other major components (Table 1.4). Such a gum product may be used as a moisturizer in pharmaceutical and cosmetic products or in food products as a thickener, stabilizer and a source of valuable soluble fiber (Redmond, 1996).

β-Glucan concentrate of acceptable yield and viscosity are usually prepared from oats or barley. When oats are used, the feed material for extraction may be bran, flakes, meal or flour. Oat bran from Hinoat cultivar, which was used as the feed material by Wood et al. (1989a) in the large scale preparation, has the highest β-glucan concentration (10.9-16.6%). Such a β-glucan concentration is substantially higher than that in dehulled Hinoat (4.7-5.7%). β-Glucan from barley may be extracted directly from flour or bran. When Bhatty (1992a) used bran separated from dry-milled barley kernels, he obtained a 1.36 fold enrichment of β-glucan in the bran prior to extraction.

1.3.1. Preparation of samples

1.3.1.1. Grinding

Samples of oats and barley are usually ground to pass screens of different size openings in order to increase the contact area for the extraction solvent. Wood et al. (1978) varied the size of openings (0.5-3 mm) to study the effect of particle size. They obtained better yield of β-glucan with smaller particle size;

Table 1.4. Composition of β-glucan preparation isolated from barley (Tupper and Azhul varieties) and oat bran with 20% Na_2CO_3 (%, as is)[1].

Component	Tupper bran	Azhul bran	Oat bran
β-Glucan	57.9	71.9	79.7
Protein	3.1	3.1	3.1
Starch	1.1	0.4	1.2
Ash	9.5	3.9	3.7
Pentosans	7.8	2.9	1.1

[1] Adapted from Bhatty (1993).

4.07% for 0.5 mm and 3.32% for 3 mm openings. Generally, 500 μm was the smallest particle size investigated. After grinding barley to 250 μm and sieving to separate into different size fractions, Knuckles et al. (1992) found that the fraction with particle size >147 μm had the highest β-glucan content, while the <64 μm fraction was enriched in starch. Bhatty (1992a) found higher β-glucan content in coarser particles (>236 μm) of bran and shorts. It is obvious that larger particles rich in β-glucan may be extracted more effectively if they are ground to a smaller size, especially when extraction time is short (0.5 h). The smaller particle size can also influence the separation of protein and starch fractions, but this effect was not investigated.

Ground barley samples were stored at 5°C (Bhatty et al., 1991) or at -17°C (Wood et al., 1989a) if they were not used immediately. There was no attempt to study the effect of cooling and freezing of barley flour on the extract viscosity and yields. However, no significant changes are anticipated under refrigeration if the moisture content of the barley flour is sufficiently low (<12%) since the enzyme activity is negligible.

1.3.1.2. Inactivation of enzymes by ethanol reflux

The role of endo-enzymes in barley is to breakdown cell walls and allow access to starch reserves in the seed. Endo-(1→4)-β-D-glucanase presence is due to microbial contamination, while endo-(1→3)-β-D-glucanase and endo-(1→3)(1→4)-β-D-glucanase are constitutive to germinating barley grain (Brunswick et al., 1987). Brunswick et al. (1987) also found significantly

($p<0.05$) higher activity for endo-(1→3)-β-D-glucanase in imbibed barley compared to that of endo-(1→3)(1→4)-β-D-glucanase. Enzyme activity was assessed by viscometry. Kilning at 60°C for 16 h decreased endo-(1→3)(1→4)-β-D-glucanase activity by 70-80% whereas endo-(1→3)-β-D-glucanase activity was reduced only 30%. Ballance and Meredith (1976) found that endo-(1→3)-β-D-glucanase was stable at pH 4.75 and at pH 8.5. Optimal pH for endo-(1→3)-β-D-glucanase was 5.0 on a laminaran substrate and pH 5.8-5.9 on carboxymethyl pachiman. The endo-(1→3)-β-D-glucanase was stable at 60°C for 40 min, but when the temperature was increased to 70°C its activity level was negligible. However, some barley β-glucan endo-hydrolases are quite heat labile and are inactivated at lower temperatures. The isoelectric point (pI) for endo-(1→3)-β-D-glucanase is 9.8 (Ballance and Meredith, 1976).

Enzymes of the glucanase type are useful in malting and brewing because BBG is a source of problems throughout the brewing process. Due to its high viscosity, BBG can slow down filtration, heat transfer and clarification of beer. Reacting with tannins, β-glucan can form haze in beer, which is highly undesirable. However, BBG improves the taste and foam stability of the final product (Pikus, 1995).

β-Glucan solubilase is another enzyme present in barley that can be considered very useful. Bamforth et al. (1979) found that this enzyme has increased activity at temperatures as high as 80°C with optimal activity at pH ~6.35. β-Glucan solubilase was more heat stable than enzymes of the glucanase

type since it retained 60% of its activity after 30 min at 73°C. Bamforth et al. (1979) concluded that the activity of endo-β-glucanase in steeped barley is negligible and that the initial catabolism of β-glucan is due to solubilase activity. This is in contrast to the findings of Brunswick et al. (1987) who found considerable endo-(1→3)-β-D-glucanase activity in imbibed barley. However, β-glucan solubilase may have a significant role in yields of β-glucan gums from nonrefluxed flour. Endo-β-glucanases must be inactivated if high viscosity β-glucan gum is to be obtained from barley.

Anderson et al. (1978) reviewed the different approaches used to inactivate β-glucanases prior to β-glucan extraction (Table 1.5). Reflux with 80% ethanol has been the most common approach. However, these studies failed to check the residual activity of enzymes after reflux. Preece and McKenzie (1952) were the first to use refluxing with 80% ethanol for enzyme inactivation. This technique has been adopted by other researchers since then. In addition, Preece and McKenzie (1952) developed the purification procedure for β-glucan using precipitation with ammonium sulfate. In spite of refluxing, Wood et al. (1989a) noticed a decline in the viscosity of the oat extract. They hypothesized that it might be because of residual enzyme activity of fungal origin or nonenzymic -cleavage induced by phenolics in alkaline solution.

Heat pretreatment of raw material for the inactivation of enzymes in addition to the use of a higher pH (pH 10) for extraction was suggested by Wood et al. (1978). Recently, this approach has been adopted for the development of a commercial oat product which is stable at room temperature for a year (Redmond,

Table 1.5. Summary of methods for barley β-glucan extraction[1].

β-glucanase inactivation	Extraction	Fractionation	Reference
80% EtOH[2] reflux 2x30 min	water 40°C 3x3 min.	$(NH_4)_2SO_4$	Preece and McKenzie (1952)
85% EtOH 30 min.	papain/water at 20°C, 2h, papain ppt by T.C.A.	$(NH_4)_2SO_4$	Bass and Meredith (1955)
T.C.A. 4.4% at 70°C, pH 1, 3 min.		amylase digestion, acetone ppt. 2 vol.	Luchsinger et al. (1958)
85% EtOH 30 min.	papain/water at 20°C, 2 h, papain ppt by T.C.A.	95% ethanol w/v	Bass and Meredith (1959)
not inactivated	water at room temp. 2 h	95% ethanol w/v	Bass and Meredith (1959)
85% EtOH 60 min.	papain/water at 20°C, 2 h, papain ppt by T.C.A.	amylase digestion, T.C.A. ppt to remove amyl., acetone ppt 1vol, $(NH_4)_2SO_4$ ppt	Schuster et al. (1967)
85% EtOH 30 min	papain/water at 40°C	$(NH_4)_2SO_4$	Sparrow and Meredith (1969)
80% EtOH 2x30 min	water at 65°C, 3 extr. Over 22 h	amylase digestion, 20% $(NH_4)_2SO_4$	Bourne and Pierce (1970)
papain/amylase at 65°C		acetone ppt., 30% $(NH_4)_2SO_4$	Scott (1972)
80% EtOH 1 h	water at 40°C 3x30 min	filtration, dialysis	Fleming et al. (1974)
70% EtOH at 100°C	water at 65°C, 4% NaOH at 20°C	NA	Palmer (1975)
1) boil. 80% EtOH 1 h 2) boil. 80% EtOH 1 h 3) boil. 80% EtOH 1 h add water heated at 100°C (10 min.) α-amylase (overnight, room temp.) heated 100°C 30 min.	water 40°C 3x30 min water 65°C 3x30 min. water 100°C 2x30 min.	filtration, dialysis 20% $(NH_4)_2SO_4$ reppt. Acetone 4 vol.	Fleming and Kawakami (1977)

[1] Adapted from Anderson et al. (1978).

[2] Vol./vol. concentration for all ethanol samples.

1996). However, ethanol refluxing of the feed material has some drawbacks for commercial scale production. It is expensive, time consuming and demands an additional enzyme treatment of the extract because of starch solubilization.

1.3.2. Extraction

Extraction is the main step of the overall procedure. Yield, purity, stability and viscosity of the gum product are determined by the following extraction parameters:

a) solvent-to-feed ratio,

b) type of solvent,

c) pH of extraction,

d) temperature of extraction, and

e) the number of extraction steps.

Optimization of the above extraction parameters should take into account their interaction effects. In addition, other factors such as the speed of stirring or shear rates during centrifugation may influence the quality of the gum, especially in large scale operations.

1.3.2.1. Solvent-to-feed ratio

For technical, environmental and economical reasons it is desirable to use the least possible amount of solvent. Wood et al. (1978) did not find a difference in the yield of oat gums extracted with a 10:1 versus a 20:1 solvent : flour ratio. For large scale extraction, a higher ratio is probably more desirable for ease of pumping and centrifugation. Wood et al. (1989a) applied a 20:1 ratio for pilot

plant extraction of oats whereas Bhatty (1995) used a 50:1 ratio when he extracted barley β-glucans with NaOH.

1.3.2.2. Type of solvent and pH

The most commonly used solvents for β-glucan extraction are water, Na_2CO_3 and NaOH. In addition, an acidic buffer (82.8 ml of 1N HCl and 7.46 g of KCl, pH 1.5) was used for the testing of acid extract viscosity (AEV) (Bhatty, 1992b). Although water extracts had higher yields of β-glucan than acid extracts (1.8% vs. 1.1%), their viscosity was lower (4.0 vs. 18.2 cSt) (Bhatty, 1992b). This result is expected because the pH of a water extract is ~6, which is very favorable for the activity of both β-glucan solubilase and endo-β-(1→3)-glucanase enzymes. Bhatty (1993) studied the effect of the type of solvent and pH on yield by testing sodium carbonate at pH 7 and 10 versus 4% NaOH at different temperatures. It was clearly shown that the extraction at pH 10 is advantageous over that at pH 7 in terms of β-glucan recovery (55 vs. 40%) and viscosity (>70 cSt vs. <20 cSt). As well, NaOH produced the highest recovery (81%), but purity (59.7 vs. 71.9% for pH 10 extract) and viscosity (<20 cSt) were lower. The lower purity was due to the enhanced extraction of pentosans and ash. This was expected since NaOH is a common solvent for the extraction of pentosans and hemicelluloses from cereals. In addition, too high a pH of 4% NaOH (even though not reported, it is expected to be ~13) is favorable for the cleavage of β-glucan because its 1→3 bond is susceptible to alkali degradation (McCleary and Codd, 1991). Consequently, a less viscous product is obtained which is in contrast to the usual goal of β-glucan extraction. High extraction pH enhances protein digestion and

thus helps the release of β-glucan from the protein matrix it is entrapped in within the cell walls. Use of strong alkali has drawbacks if protein and starch fractions are desired products in addition to β-glucan, since high pH leads to the degradation of proteins (Hurrell and Pinot, 1985). As well, working with strong alkali may be dangerous since even a small spill can damage equipment or harm laboratory personnel.

Other solvents that were tried for β-glucan extraction caused additional problems. For example, Wood et al. (1978) used a sodium carbonate - sodium bicarbonate buffer which caused foaming during neutralization and a delay in the procedure.

In conclusion, a solution of sodium carbonate is the preferred solvent for β-glucan extraction for food uses. However, the extraction pH needs to be optimized. Any pH that will enhance the inactivation of glucanase enzymes is preferred. But pH alone (≤10) is not sufficient to inactivate these enzymes. Indeed, Wood et al. (1978) confirmed that active enzymes are still extracted at pH 10. Obviously, an additional factor is needed to ensure inactivation. A heat treatment of either raw material (i.e. steaming) or β-glucan extract seems to be the best approach.

1.3.2.3. Temperature of extraction

Several researchers showed that the extraction yield is proportional to the temperature of extraction (Wood et al., 1978, 1991; Dawkins and Nnanna, 1993). While Wood et al. (1978, 1991) used extraction temperatures of 45, 65 and 80°C, Dawkins and Nnanna (1993) studied a narrower range of 50, 55, 60 and 70°C in

the range of starch gelatinization temperatures. Starch gelatinization during β-glucan extraction needs to be minimized since starch and protein are the main components which decrease gum purity. As expected, 50-55°C range was the most desirable for the extraction of highest purity β-glucan. However, other tests to study the effect of extraction temperature on viscosity and stability of these gums were not performed.

The optimum temperature should be the highest possible temperature below the temperature of starch gelatinization. Since starch gelatinization temperature can vary from 51.1°C to 68°C depending on other parameters (MacGregor and Fincher, 1993), it must be determined experimentally for each barley cultivar. The combined effect of pH and temperature determines the extent of protein solubilization.

1.3.2.4. Number of extraction steps

It was noted that oats and barley behave differently during subsequent extractions. While it was possible to achieve about 80% recovery of soluble β-glucan in the first extraction step of barley as shown in Figure 1.2, this amount was less than 50% with oats (Wood et al., 1977). Similar results were reported for oats by Wood et al. (1978). Therefore, for a large scale single step extraction of β-glucan, barley seems to be a better source of this fiber than oats.

1.3.2.5. Time of extraction

In a large scale process, the aim is to extract the largest amount of β-glucan in the shortest possible time. A longer extraction time will require more vessels thereby adding to the cost while a shorter time may result in a smaller

Page 21 has been removed due to copyright restrictions. The information removed was Figure 1.2 which describes the distribution of gum (expressed as a percentage of the total extracted) between successive extractions of oats and barley (Fig. 2 in Wood et al., 1977).

yield. Thus, it is important to optimize extraction time to achieve maximum yield. While Wood et al. (1977, 1978, 1989a) preferred a 0.5 h extraction, Dawkins and Nnanna (1993) and Bhatty (1995) applied 1h extraction times.

1.3.3. Purification of the extract

At the end of the extraction period, a two phase mixture of soluble and insoluble substances is obtained. Starch, proteins, fibers, cellulose, minerals etc. are distributed between the two phases in a ratio which depends on the properties of the raw material and extraction conditions. Separation of the above fractions usually requires the following steps:

a) separation of insolubles,

b) separation of solubles, and

c) additional purification (optional).

1.3.3.1. Separation of impurities

The easiest way to separate the insolubles is by sedimentation, but this natural process is too slow. The addition of centrifugal force speeds up the process and gives better separation of solids. The force applied depends on the amount of sample, difficulty of separation and the available equipment. Higher centrifugal forces can be achieved with laboratory scale equipment compared to continuous centrifuges used in a pilot plant extraction.

In the laboratory scale extraction of oats, Autio et al. (1987) and Dawkins and Nnanna (1993) used 16,300 x g to separate the solids rich in starch after which proteins were separated. Prior to the second centrifugation the pH of the supernatant should be adjusted to 4.5, which is the isoelectric point of some

proteins. In a laboratory scale extraction the centrifugal force applied to separate proteins is usually higher than that used for the first step for insolubles; it may reach 21,000 x g (Wood et al., 1977).

Both starch and protein have been separated at lower speeds in pilot plant procedures due to equipment limitations. Wood et al. (1989a) used 5,000 x g and 8,400 x g for starch and protein separations from oat extract, respectively. Similarly, Bhatty (1995) employed a centrifugation force of 6,460 x g for both starch and protein fractions for 15 min. The time of centrifugation usually varies between 15 - 20 min. During centrifugation, the working temperature was usually room temperature. Sometimes a higher temperature of 50°C was preferred (Bhatty, 1995) in order to decrease the viscosity and ease the flow of a solution through a continuous centrifuge. Rarely was temperature decreased to rather low values where the extract is purified at 4°C (Wood et al., 1977). Such a low temperature is applicable only for a laboratory scale extraction since increased viscosity actually makes separation harder but decreases enzyme activity significantly.

1.3.3.2. Additional purification

Further purification of the extract after the removal of starch and proteins is not very common. However, if a harsh extraction procedure with 0.25 - 1 N NaOH is performed, solubilization of a substantial amount of starch can result in a lower purity gum (Bhatty, 1995). In this case, the starch should be digested with the addition of a thermostable α-amylase (Termamyl 120 LN) at elevated temperatures 80 - 96°C. This procedure has a dual goal. Beside starch digestion, it

also inactivates residual glucanase enzymes present in the extract. Purification with pancreatic α-amylase and amyloglucosidase was also reported (Saulnier et al., 1994), but in this case the objective was the characterization of pure β-glucan, not the gum per se.

1.3.4. Precipitation of gum

In general, β-glucan is precipitated from the extract solution with the addition of an alcohol. Isopropyl alcohol (IPA) and ethanol are the most commonly used alcohols. Both are added at ~100% purity in equal volume to supernatant, so that the final concentration is 50% (v/v). The potential toxicity of IPA makes ethanol the preferred choice. In earlier reports (Wood et al., 1977, 1978), IPA was used as the precipitating agent. More recently, with the pilot plant production of β-glucan (Wood et al., 1989a; Bhatty, 1995) and consideration of potential use of the β-glucan product in food systems, scientists have switched to ethanol.

The precipitated gum in alcohol solution may be left to sit overnight at 5°C (Wood et al., 1977, 1978; Bhatty, 1995) which may be beneficial for the complete removal of water from pockets inside gum flakes. Remnants of residual water produce a gum which is not fluffy since a hard crust layer is formed during air drying of the gum as the water diffuses out and evaporates from the surface.

Precipitated gum may be centrifuged, homogenized in alcohol and air dried as it was performed by Wood et al. (1977, 1978, 1989a) and Dawkins and Nnanna (1993). This procedure is certainly easier and simpler than additional

resuspending in water and freeze drying as performed by Bhatty (1993, 1995) and Saulnier et al. (1994).

1.4. FUNCTIONAL PROPERTIES OF β-GLUCAN

1.4.1. Viscosity

The viscosity of barley extracts depends on a number of factors. The year and location of the barley crop play a role in its β-glucan content and viscosity. Hot and dry conditions during the grain filling period result in the highest β-glucan content and viscosity (Ulrich et al., 1986). Varietal influence is of utmost importance as well. Extracts of waxy varieties have higher viscosity than those from normal starch varieties. Bhatty (1992a) not only observed that waxy varieties have a higher β-glucan content, but also that they differ among themselves in AEV which may be medium or high viscosity (Table 1.6).

Bhatty et al. (1991) stated that upon hydrolysis with β-glucanase, protease, xylanase and α-amylase, the single factor contributing most to the viscosity was β-glucan with a small influence of pentosans (Figure 1.3). Similar findings were presented by Autio et al. (1992) where the addition of purified endo-β-glucanase brought the viscosity of β-glucan extract to that of water.

β-Glucan solutions exhibit pseudoplastic behavior (Wood et al., 1978; 1989a; Autio et al., 1987; Bhatty et al., 1991; Dawkins and Nnanna, 1995) and their behavior can be described by the power law model

$$S = c\,R^{n}$$

Table 1.6. β-Glucan and acid extract viscosity (AEV) of normal and waxy starch lines of barley[1].

Genotype	Starch	β-glucan (%)	AEV (cSt)	Description
SB87697	Normal	4.1	5.4	Average β-glucan,
SB88490	Normal	3.8	4.4	low viscosity
SB85740	Waxy	6.9	55.0	High β-glucan,
SB85751	Waxy	7.0	51.3	medium viscosity
SB85738	Waxy	7.2	289.9	High β-glucan,
SB85745	Waxy	6.9	275.9	high viscosity

[1] From Bhatty (1992a).

Page 27 has been removed due to copyright restrictions. The information removed was Figure 1.3 which describes the effect of sequential addition of xylanase and β-glucanase on acid extract viscosity of hulless barley (Fig. 4 b in Bhatty et al., 1991).

where S is shear stress (N/m^2), R is shear rate (s^{-1}) and c and n are constants for the consistency coefficient and flow behavior index, respectively. For pseudoplastic solutions, value of the flow behavior index is $0 < n < 1$. The smaller the value for the flow behavior index, the greater is the shear sensitivity. An increase in the concentration of β-glucan causes the consistency coefficient to increase and the flow behavior index to decrease, indicating that the solution becomes more pseudoplastic (Autio et al., 1987) as presented in Table 1.7 for oat β-glucan. Such information is necessary to predict the behavior and mouthfeel of gum solutions. Szczesniak and Farkas (1962) reported that gums with lower n have a cleaner mouthfeel, while gums with higher n values tend to feel slimy in the mouth. An exponential increase in viscosity with concentration is presented in Figure 1.4 (Bhatty et al., 1991).

Pseudoplasticity also means that viscosity decreases with shear rate (Figure 1.5). Wood et al. (1989a) observed that gum solutions exposed to high shear rates during purification in a pilot plant may have lower viscosity when compared to gums obtained under the same conditions at the bench scale level of production. The viscosity of bench gum was reported to be three times higher than that for pilot plant gums (Wood et al., 1989b). This phenomenon is the result of polymer chain breakage which usually occurs in the proximity of the chain center (Harrington and Zimm, 1965). Ultrasonication may also reduce the viscosity of polysaccharide solutions due to the high shear force involved (Szu et al., 1986).

Table 1.7. Power law constants for oat β-glucan in water and in 25% sucrose solution at 25°C[1].

β-glucan powder conc. (%, w/w)	Flow behavior index		Consistency coefficient	
	n_w[a]	n_s[b]	c_w[a] (N x s^n/m^2)	c_s[b] (N x s^n/m^2)
0.20	0.84	0.83	0.04	0.06
0.45	0.62	0.53	0.57	1.49
0.67	0.50	0.40	3.40	7.28
0.89	0.30	0.29	12.2	21.3
1.00	0.28	0.26	24.2	31.2
1.10	0.24	0.23	36.1	40.3
1.22	0.20	0.21	46.9	50.7

[1] From Autio et al. (1987).
[a] β-Glucan in water.
[b] β-Glucan in 25% sugar solution.

Page 30 has been removed due to copyright restrictions. The information removed was Figure 1.4 which describes the relationship between β-glucan concentration and viscosity of acid buffer extracts (Fig. 5 in Bhatty et al., 1991).

Page 31 has been removed due to copyright restrictions. The information removed was Figure 1.5 which describes the effect of shear rate on viscosity of oat gum (Fig. 4 in Dawkins and Nnanna, 1995).

1.4.1.1. Effect of temperature

In general, viscosity of liquids decreases with temperature; this is also the case for β-glucan solutions as presented in Figure 1.6 (Dawkins and Nnanna, 1995). The fact that this temperature effect is reversible may be a very desirable property for potential applications of β-glucan in desserts such as ice cream and frozen yogurt where high cold viscosity gives good texture and mouthfeel, while low hot viscosity enables more efficient processing i.e. heating and pumping. These results are in agreement with the findings in our laboratory (Temelli, 1994).

1.4.1.2. Effect of pH

Dawkins and Nnanna (1995) confirmed the theoretical postulate that neutral and nonionic polymers behave independent of pH (Figure 1.7). Oat β-glucan solutions were stable in the pH range 2 - 10. This trait makes the application of β-glucan gum in various food products possible.

1.4.1.3. Effect of NaCl

Conflicting results have been reported on the effect of salt on viscosity. Autio et al. (1987) found no influence of salt on the viscosity of oat β-glucan gum in the concentration range 0 - 10% (w/w), whereas Dawkins and Nnnanna (1995) reported a slight increase in viscosity at low salt concentrations (0.1 - 0.2%) and a rather pronounced decrease at higher NaCl concentrations (Figure 1.8). The viscosity increase was explained based on the higher solubility of protein impurities in the presence of a small amount of NaCl while the decrease in viscosity was the consequence of less water being available for the hydration of the gum.

Page 33 has been removed due to copyright restrictions. The information removed was Figure 1.6 which describes the effect of temperature on oat gum viscosity (Fig. 5 in Dawkins and Nnanna, 1995).

Page 34 has been removed due to copyright restrictions. The information removed was Figure 1.7 which describes oat gum viscosity as a function of pH (Fig. 6 in Dawkins and Nnanna, 1995).

Page 35 has been removed due to copyright restrictions. The information removed was Figure 1.8 which describes the effect of NaCl on oat gum viscosity (Fig. 7 in Dawkins and Nnanna, 1995).

1.4.1.4. Effect of sucrose

The addition of sucrose at a level of 25% (w/w) (Autio, 1988) or in the concentrations ranging 20 - 45% (Dawkins and Nnanna, 1995) increased the viscosity of oat β-glucan. A similar result was reported by Autio et al. (1987) when they added 25% and 50% (w/w) of sugar to β-glucan solution. However, a much higher concentration of 65% (Dawkins and Nnanna, 1995) or 75% (Autio et al., 1987) restricted viscosity, which was explained by the low solubility of β-glucan in such a concentrated syrup. Elfak et al. (1977) suggested that sugars added in conjunction with higher polymer concentrations restrict the hydration and extension of polymer molecules.

1.4.2. Gelation of β-glucan

The literature lacks information on the gel behavior of β-glucan. Only Doublier and Wood (1995) observed that partially hydrolyzed oat gum exhibits gel-like behavior. They rightfully supposed that the higher viscosity of gum prevents chains of β-glucan from approaching each other and forming a firmer bound network.

Several factors may have contributed to the failure to investigate the gelling behavior of β-glucan. The ultimate goal of β-glucan extraction has been to obtain a high viscosity gum which can compete with other hydrocolloids in functional properties. As well, the health benefits of β-glucan such as the hypocholesterolemic effect and the blood glucose regulating ability are directly correlated with the high viscosity of β-glucan (Malkki et al., 1992). In addition,

the high price of industrial β-glucan (about $1000/kg) has forced researchers to focus on achieving the highest viscosity using the smallest possible β-glucan concentration. However, it would be impossible to form a gel at low β-glucan concentrations.

1.4.3. Foam and emulsion stabilizing properties of β-glucan

There are no reports in the literature describing the foam and emulsion stabilizing properties of β-glucan. However, previous findings in our laboratory show that barley β-glucan does stabilize a foam and emulsion at a level comparable to or better than guar and arabic gums (Temelli, 1995). Thus, BBG show potential for use as a stabilizer. The high viscosity of these gum solutions certainly contributes to their foam and emulsion stabilizing effect. Figure 1.9 gives a comparison of the viscosity of oat gum to that of other commonly used food hydrocolloids. Similar results were reported by Temelli (1995) for viscosity of BBG gum compared to guar and arabic gums at higher shear rates.

1.5. FOOD APPLICATIONS OF β-GLUCAN GUM

The literature is lacking on the β-glucan applications in food systems and interactions of β-glucan with other food components such as proteins, lipids, various salts etc. or industrial uses of β-glucan. Potential uses of high β-glucan cereals and their derivatives as a supplement in baked products like bread, muffins, pancakes and pastas were discussed by Newman and Newman (1991) and Hawrysh et al. (1996).

Page 38 has been removed due to copyright restrictions. The information removed was Figure 1.9 which compares viscosity of oat gum with food grade gums (Fig. 9 in Dawkins and Nnanna, 1995).

The potential for wider application of β-glucan certainly exists since oat β-glucan production has already reached commercialization and has some industrial applications. Oat β-glucan produced at the demonstration plant of Canamino Inc. (Saskatoon, SK) has been incorporated into some prototype food products such as beverages and imitation ice cream product for lactose intolerant persons and udon noodles that are to be marketed in Japan. Oat β-glucan also found an application in the cosmetic and animal care products such as "organic" or "bio" shampoos, creams and ointments (Redmond, 1996). However, any information on β-glucan behavior in the presence of other food ingredients has been kept confidential. The large scale marketing of the above products has been limited by the relatively small production capacity of Canamino Inc.

Another commercial product that has been incorporated into numerous food products as a fat substitute is Oatrim®. Oatrim® is an oat derivative produced by hydrolysis of a slurry made of roasted oat flour, amylase enzymes and water (Jenkins, 1996). β-Glucan is present as a minor component in Oatrim® and its concentration varies between 1-10% (w/w). Thus, it is impossible to predict the influence of β-glucan on the properties of Oatrim® because ~90% of the components are dextrins which also exhibit similar water binding and fat replacing properties. However, reported health benefits of Oatrim® have been attributed to its β-glucan content (Hallfrich et al., 1995).

1.6. OBJECTIVES OF THESIS

β-Glucan concentrate shows great potential for application in various food products. It exhibits functional characteristics similar to or better than some commonly used hydrocolloids. Recovery and viscosity characteristics of oat β-glucan have been studied to a greater extent than that of BBG. In fact, the production of oat β-glucan has reached commercialization and oat gum is being marketed. Currently, the main obstacle for its wider usage is its high price due to its limited production from oats. Barley β-glucan show similar potential but they have not been studied to the same extent as oat β-glucan. Considering the potential for large scale preparation from both barley and oats, scientific and technological advances made in the recent years, health benefits associated with β-glucan and independence from fluctuating export markets, one can only expect that the era of β-glucan is yet to come. For this to become reality, further investigation of the recovery of barley β-glucan, their functional properties and interaction with other food components is essential. Thus, the objectives of this thesis are:

(a) to investigate the effect of extraction conditions and different thermal treatments (reflux of flour, boiling and purification of extract with thermostable α-amylase) on the yield, purity and viscosity stability of BBG gum (chapter 2),

(b) to study the interactions of β-glucan with different salts and dairy components in order to assess the potential for β-glucan applications in food products (chapter 3), and

(c) to determine whether BBG gum can form a gel and if it can, to characterize the behavior of β-glucan gel (chapter 4).

1.7. REFERENCES

American Diabetes Association, 1994. Position statement. Nutrition recommendations and principles for people with diabetes mellitus. J. Am. Diet. Assoc. 94: 504-506.

Anderson, J.W. 1980. Dietary fiber and diabetes. In: *Medical Aspects of Dietary Fiber*. G.A. Spiller and R.M. Kay (Ed.). Plenum Medical Book Co., New York.

Anderson, M.A., Cook, J.A., and Stone, B.A. 1978. Enzymatic determination of 1,3:1,4-β-glucans in barley grain and other cereals. J. Inst. Brew. 84: 233-239.

Autio, K. 1988. Rheological properties of solutions of oat β-glucans. In: *Gums and Stabilizers for the Food Industry 4.* G. O. Phillips, P. A. Williams and D. J. Wedlock (Ed.), p. 483-488, IRL, Washington, D C.

Autio, K., Myllymaki,O., and Malkki, Y., 1987. Flow properties of solutions of oat β-glucans. J. Food Sci. 52(5): 1364-1366.

Autio, K., Malkki, Y., and Virtanen, T. 1992. Effects of processing on the microstructure of oat (Avena sativa) bran concentrate and the physicochemical properties of isolated β-glucans. Food Structure, 11: 47-54.

Ballance, G.M., and Meredith, W.O.S. 1976. Purification and partial characterization of an endo-β-1,3-glucanase from green malt. J. Inst. Brew. 82: 64-67.

Bamforth, C.W., Martin, H.L., and Wainwright, T. 1979. A role of carboxypeptidase in the stabilization of barley β-glucan. J. Inst. Brew. 85: 334-338.

Bass, E.J., and Meredith, W.O.S. 1955. Enzymes that degrade barley gums. III. Studies of beta-polyglucosidases of green malt. Cereal Chem. 32: 374-381.

Bass, E.J., and Meredith, W.O.S. 1959. Proc. Am. Soc. Brew. Chem. p. 129. Cited in Anderson, M.A., Cook, J.A., Stone, B.A. 1978. Enzymatic determination of 1,3:1,4-β-glucans in barley grain and other cereals. J. Inst. Brew. 84: 233-239.

Beresford, G. and Stone, B.A. 1983. (1→3)(1→4)-β-Glucan of Triticum grains. J. Cereal Sci. 1: 111-114.

Bhatty, R.S. 1986. Physicochemical and functional (breadmaking) properties of hull-less barley fractions. Cereal Chem. 63(1): 31-35.

Bhatty, R.S. 1992a. β-Glucan content and viscosities of barleys and their roller milled flour and bran products. Cereal Chem. 69(5): 469-471.

Bhatty, R.S. 1992b. Total and extractable β-glucan contents of oats and their relationship to viscosity. J. Cereal Sci. 15: 185-192.

Bhatty, R.S. 1993. Extraction and enrichment of (1→3)(1→4)-β-D-glucan from barley and oat brans. Cereal Chem. 70(1) : 73-77.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22: 163-170.

Bhatty, R.S., MacGregor, A.W., and Rossnagel, B.G. 1991. Total and acid soluble β-glucan content of hulless barley and its relationship to acid extract viscosity. Cereal Chem. 68(3): 221-227.

Bourne, D.T. and Pierce, J.S. 1970. β-Glucan and β-glucanase in brewing. J. Inst. Brew. 76: 328-335.

Brunswick, P., Manners, D.J., and Stark J.R. 1987. Development of β-D-glucanases during germination of barley and the effect of kilning on individual isoenzymes. J. Inst. Brew. 93: 181-186.

Chen, W-J.L., Anderson, J.W., and Jennings, D. 1984. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol - fed rats. Proc. Soc. Exp. Biol. Med. p. 175-215.

Chung, O.K. and Pomeranz, Y. 1985. Amino acids in cereal proteins and protein fractions. Ch. 5 in *Digestibility and Amino Acid Availability in Cereals and Oilseeds.* J.W. Finley and D. T. Hopkins (Ed.), p. 169-232. AACC, St. Paul, Minnesota.

Dawkins, N.L. and Nnanna, I.A. 1993. Oat gum and β-glucan extraction from oat bran and rolled oats: temperature and pH effects. J. Food Sci. 58: 562-566.

Dawkins, N.L. and Nnanna, I.A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9(1): 1-7.

Doublier, J.L. and Wood, P.J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats. Cereal Chem. 72(4): 335-340.

Elfak, A.M., Pass, G., Philips, G.O., and Morley, R.G. 1977. The viscosity of dilute solutions of guar gum and locust bean gum with and without added sugars. J. Sci. Food Agric. 28: 895-899.

Fastwood, M. 1987. Dietary fiber and the risk of cancer. Nutr. Rev. 45: 193.

Fleming, M., and Kawakami, K. 1977. Studies of the fine structure of β-D-glucans of barley extracted at different temperatures. Carbohyd. Res. 57: 15-23.

Fleming, M., Manners, D.J., Jackson, R.M., and Cooke, S.C. 1974. The estimation of β-glucan in barley. J. Inst. Brew. 80: 399-404.

Gosain, K. 1996a. Long Term Effects of Barley Bread Products on Metabolic Control of Non Insulin Dependent Diabetes Mellitus. MSc. Thesis, University of Alberta, Edmonton.

Gosain, K. 1996b. Personal communication. Alberta Barley Commission. Calgary, AB.

Hallfrich, J., Scholfield, D., and Behall, K.M. 1995. Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. Am. J. Clin. Nutr. 61: 379-384.

Harrington, R.E., and Zimm, B.H. 1965. Degradation of polymers by controlled hydrodynamic shear. J. Phys. Chem. 69:161-175.

Hawrysh, Z.J., Pick, M.E., Gee, M.I., and Brudler, J. 1996. Sensory and instrumental evaluation of stored waxy hulless barley bread. Abstract # 80B-10. Presented at the 57th Annual Meeting of IFT, New Orleans, LA. June 22-26.

Hurrel, R.F., and Finot, P.A. 1985. Effects of food processing on protein digestibility and amino acid availability. Ch. 8 in *Digestibility and Amino Acid Availability in Cereals and Oilseeds.* J.W. Finley and D.T. Hopkins (Ed.) p. 233-246. AACC, St. Paul, Minnesota.

Ilmann, R.J., and Topping, D.L. 1985. Effects of dietary oat bran on faecal excretion, plasma volatile fatty acids and lipid synthesis in rats. Nutr. Res. 5: 839.

Jenkins, R. 1996. Personal communication. Rhone Poulenc Co. Washington, PA.

Kahlon, T.S., Chow, F.I., Knuckles, B.E., and Chiu, M.M. 1993. Cholesterol-lowering in hamsters of β-glucan-enriched barley fraction, dehulled whole

barley, rice bran, and oat bran and their combinations. Cereal Chem. 70(4): 435-440.

Knuckles, B.E., Chiu, M.M., and Betschart, A.A. 1992. β-glucan enriched fractions from laboratory scale dry-milling and sieving of barley and oats. Cereal Chem. 69(2): 198-202.

Luchsinger, W.W., English, H., Cochrane, D.G., and Kneen, E. 1958. Proc. Am. Soc. Brew. Chem. p. 40. Cited in Anderson, M.A., Cook, J.A., and Stone, B.A. 1978. Enzymatic determination of 1,3:1,4-β-glucans in barley grain and other cereals. J. Inst. Brew. 84: 233-239.

MacGregor, A.W., and Fincher, G.B. 1993. Carbohydrates of the barley grain. Ch. 3 in *Barley: chemistry and technology.* A.W. MacGregor and R.S. Bhatty (Ed.) p. 73-130. AACC, St. Paul , Minnesota.

Malkki, Y., Aautio, K., Hanninen,O., Myllimaki, O., Pelkonen, K., Suortti, T., and Torronen, R. 1992. Oat bran concentrates: Physical properties of β-glucan and hypocholesterolemic effects in rats. Cereal Chem. 69(6): 647-653.

McCleary, B.V. and Codd, R. 1991. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55: 303-312.

Miller, S.S. and Fulcher, R.G. 1994. Distribution of (1→3),(1→4)-β-D-glucan in kernels of oats and barley using microspectrofluorometry. Cereal Chem. 71(1): 64-68.

National Research Council. 1979. *Nutrient Requirements for Domestic Animals*. 8th. ed. National Academy Press, Washington, DC.

Newman, R.K. and Newman, C.W. 1991. Barley as a food grain. Cereal Foods World 36: 800-805.

Newman, R.K., Klopfenstein, C.F., Newman, C.W., Guritno, N., and Hofer, P. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran and wheat red dog in chicks and rats. Cereal Chem. 69(3): 240-244.

Palmer, G.H. 1975. Influence of endosperm structure on extract development. Proc. Am. Soc. Brew. Chem. 33: 174-180.

Pick, M. 1994. Oat Bran Concentrate Bread Products: Long Term Effects On Diabetic Control. MSc. Thesis, University of Alberta, Edmonton.

Pikus, W. 1995. Personal communication. Molson Brewery, Edmonton, AB.

Poehlman, J. M. 1985. Adaptation and distribution. In: *Barley*. D.C. Rasmusson, (Ed.) p. 2-17. American Society of Agronomy, Madison, WI.

Preece, I.A., and Mackenzie, K.G.1952. Non-starchy polysaccharides of cereal grains. II. Distribution of water-soluble gum-like materials in cereals. J Inst. Brew. 58: 353-362.

Prentice, N., Babler, S., and Faber, S. 1980. Enzymic analysis of beta-D-glucans in cereal grains. Cereal Chem. 57: 198-202.

Redmond, M. 1996. Personal communication. CEAPRO Developments Inc., Edmonton, AB.

Saulnier, L., Gevaudan, S., and Thibault, J.F. 1994. Extraction and partial characterization of β-glucan from endosperms of two barley cultivars. J. Cereal Sci. 19: 171-178.

Schuster, K., Narziβ, L., and Kumada, J. 1967. Barley gums and their variation during malting and brewing. I. State of the problem and methods of analysis. Brauwissenschaft 20:125-135.

Scott, R.W. 1972. The viscosity of worts in relation to their content of β-glucan. J. Inst. Brew. 78: 179-186.

Sparrow, D.H.B., and Meredith, W.O.S. 1969. Malt cytolytic activity of barleys of diverse origins and its relation to maltability. J. Inst. Brew. 75: 237-242.

Statistics Canada. 1989. Field crop reporting series. Vol. 68(8). Statistics Canada, Ottawa, ON.

Statistics Canada. 1995. Field crop reporting series. Vol. 74(8). Statistics Canada, Ottawa, ON.

Szczesniak, A.S., and Farkas, E. 1962. Objective characterization of the mouthfeel of gum solutions. J. Food Sci. 27: 381-385.

Szu, S.C., Zon, G., Schneerson, R., Robbins, J.B. 1986. Ultrasonic irradiation of bacterial polysaccharides. Characterization of depolymerized products and some applications of the process. Carbohydr. Res. 152:7-20.

Temelli, F. 1994. Functional properties of β-glucan enriched barley fractions. Abstract # P325. Presented at the 37th Annual Conference of CIFST, Vancouver, BC. May 15-18.

Temelli, F. 1995. Effect of temperature and pH on extraction and functional properties of barley β-glucans. Abstract # 22-5. Presented at the 56th Annual Meeting of IFT, Anaheim, CA. June 3-7.

Temelli, F. and Burkus, Z. 1995. Unpublished data. Department of Agricultural Food and Nutritional Science. University of Alberta, Edmonton, AB.

Ulrich, S.E., Clancy J.A., Eslick, R.F., and Lance, R.C.M. 1986. β-glucan content and viscosity of extracts from barley. J. Cereal Sci. 4: 279-285.

U. S. Feed Grains Council. 1990. Grains Database Report. The Council, Washington, D C.

Wendorf, F., Schild, R., Hadidi, N.E., Close, A.E., Kobusiewicz, M., Wieckowska, H., Issawi, B., and Haas, H. 1979. Use of barley in the Egyptian late Paleolithic. Science 205: 1341-1347.

Wood, P.J. 1984. Physicochemical properties and technological and nutritional significance of cereal β-glucans. In: *Cereal Polysaccharides in Technology and Nutrition.* V.F. Rasper. (Ed.) p. 35-78. American Association of Cereal Chemists, Inc. St. Paul, Minnesota.

Wood, P.J., Paton, D., and Siddiqui, I.R. 1977. Determination of β-glucan in oats and barley. Cereal Chem. 54(3): 524-533.

Wood, P.J., Siddiqui, I.R., and Paton, D. 1978. Extraction of high viscosity gums from oats. Cereal Chem. 55(6): 1038-1049.

Wood, P.J., Weisz, J., Fedec, P., and Burrows, V.D. 1989a. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal. Chem. 66(2) : 97-103.

Wood, P.J., Anderson, W.J., Braaten, J.T., Cave, N.A., Scott, F.W., and Vachon, C. 1989b. Physiological effects of β-glucan rich fractions from oats. Cereal Foods World 34(10): 878-882.

Wood, P.J., Weisz, J., and Mahn, W. 1991. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68(5): 530-536.

Woodward, J.R., Fincher, G.B. and Stone, B.A. 1983. Water soluble (1→3)(1→4)-β-D-glucans from barley (*Hordeum vulgare)* endosperm. II. Fine structure. Carbohydr. Polym. 3: 207-225.

2. EFFECT OF EXTRACTION CONDITIONS ON THE YIELD, COMPOSITION AND VISCOSITY OF BARLEY β-GLUCAN GUM[1]

2.1. INTRODUCTION

The research on isolation of stable β-glucan was pioneered by Preece and Mackenzie (1952). Key extraction methodologies for barley and oat β-glucan were developed by Wood et al. (1977, 1978). More recently, Bhatty (1993) investigated the influence of different solvents on recovery and viscosity of barley and oat β-glucan gums. β-Glucan recovery from barley and oat bran was 80-85% and ~55% with NaOH and Na_2CO_3, respectively, but sodium carbonate extracts had more than three times higher viscosity. Dawkins and Nnanna (1993) tested the effect of temperature-pH conditions on extractability of oat β-glucan and found that pH 9.2/50°C and pH 10/50-55°C give the highest yield and purity. These conditions were later applied by Dawkins and Nnanna (1995) to investigate the effect of concentration, temperature, NaCl, pH and sucrose on the rheological properties of oat gum and to compare its viscosity with other food grade gums such as xanthan, locust bean, guar and arabic. Their results (Dawkins and Nnanna, 1995) suggest application of oat β-glucan gum as a thickener and stabilizer in food products and, possibly, in biomedical applications. This approach represents the current emphasis on value-added processing of cereals whereas earlier studies focused mainly on estimation and characterization of β-glucan for the needs of the brewing and feed industry.

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[1] A version of this chapter is to be submitted to the Cereal Chemistry for publication.

The efforts to scale up the β-glucan extraction procedures resulted in two pilot-plant studies (Wood et al., 1989; Bhatty, 1995). Wood et al. (1989) extracted β-glucan from oat bran deactivated with refluxing in 75% ethanol for 4 h. Sodium carbonate was used to adjust the pH to 10 and a two stage extraction was carried out for 0.5 h each at 35-43°C followed by separation of starch and proteins and precipitation of β-glucans with isopropyl alcohol (IPA). There was a great difference in the viscosities of bench prepared and pilot plant gums obtained under similar conditions. These differences were attributed to the high shear rates and residual enzyme activity of fungal or bacterial origin under the pilot plant conditions. The viscosity of the bench gum was extremely high, ~2300 cP at 1% (w/v) concentration and ~20 s^{-1} shear rate, while that of pilot plant gum was only ~1/4 of that value. Extract of deactivated bran obtained with 0.2 M sodium acetate buffer had no viscosity loss after 1 h while the extract of non-treated bran lost 24% of its original viscosity. The viscosity loss of deactivated bran extracts obtained at pH 10 was ascribed to nonenzymic changes probably involving phenolics. One hour is a fairly short period to test viscosity stability as performed by Wood et al. (1989). There could have been a significant change in viscosity if the testing period was longer. Viscosity stability tests should be continued for at least 24 h. Shelf life stability testing of the food products containing β-glucan gums should be carried out over a longer period of time. However, 24 h gives an indication about enzyme stability at this development stage.

Bhatty (1995) performed laboratory and pilot plant extraction and purification of β-glucans from hulless barley and oat bran. He used 1 N NaOH as

a solvent at room temperature for 1 h. Extracts of two subsequent steps were combined and purified with thermostable α-amylase at 96°C for 1 h. Bhatty (1995) did not detect any β-glucanase activity in any of the brans by flow injection analysis (Aastrup and Jorgensen, 1988). This result may be questionable since the bran was not inactivated and the chosen analysis method may not be sensitive enough to detect low activity levels. The only reliable method for the detection of enzyme activity is the measurement of the change in the viscosity of extract in time (McCleary, 1995). Conversely, Wood et al. (1989) found increased enzyme activity in oat bran as expected. On the other hand, strong extraction pH (probably >13) used by Bhatty (1995) denatures proteins and enzymes (Hurrel and Finot, 1985). Thus, enzyme activity may not be a problem, but (1→3) bonds can be cleaved at high pH (McCleary and Codd, 1991) resulting in lower viscosity. Indeed, in spite of the high extractability of β-glucan (> 95%), Bhatty (1995) recovered only ~75% from barley bran. The difference can be attributed to the loss of short chain β-glucans liberated at high pH. The viscosity of barley and oat gums obtained by Bhatty (1995) was lower than that for oat gum obtained by Wood et al. (1989); <200 cP versus ~600 cP at the same shear rate (20 s^{-1}), except for the gum from Scout barley which had viscosity at approximately the same level. In addition, Bhatty (1995) did not test the stability of his extracts or the thinning effect due to high shear rates in the pilot plant equipment. Exhaustive extraction of β-glucan at extremely high pH may have some drawbacks. Insoluble portion of β-glucans can be solubilized at such pH levels. However, if such a gum is incorporated into fluid products, β-glucans may precipitate due to extensive

interaction between long (1→4) bonded regions which are naturally insoluble and tend to aggregate. This may not be a problem if the gum is used as a water binding agent in solid products like sausages, patties etc.

Considering the potential application of barley β-glucan (BBG) gum in a variety of food products, the objectives of this study were: (a) to investigate the effect of extraction conditions on the yield, composition and viscosity of barley β-glucan gum, (b) to determine the effect of different treatments such as ethanol reflux, boiling and Termamyl treatment on the stability of viscosity over 24 h, and (c) to study the shear thinning behavior of BBG compared to that of a pure commercial BBG product.

2.2. MATERIALS AND METHODS

Materials

Extraction was performed on two types of hulless barley: Condor (K) with normal starch and Bly Blend with waxy starch (W) which were provided by Dr. J. Helm, Alberta Agriculture, Food and Rural Development (Lacombe, AB). Bly blend is a mixture of two experimental varieties, SB89528 and SB89497. Whole kernels were first crushed in a Magic Mill Model III Plus™ (Magic Mill, Salt Lake City, UT) and then finely ground to <250 μm using a Udy Cyclone Sample Mill (model 3010-30, Udy Corp., Fort Collins, CO.) Sodium carbonate was purchased from BDH Inc. (Toronto, ON) and a 20% (w/v) solution was prepared in distilled water. Pure ethanol (99.9%) was supplied by Commercial Alcohols Inc. (Brampton, ON). Pure gums of low (LV) and high viscosity (HV) type with

viscosity of 5.9 and 114 cSt (viscosity of 1% (w/v) solution determined with Ostwald C-type Viscometer at 30°C) and molecular weight of 137,000 and 327,000 daltons, respectively, were purchased from Megazyme Australia Pty. Ltd, (Warriewood (Sidney), NSW, Australia). Termamyl 120 LN (thermostable α-amylase from *Bacillus licheniformis*, E.C. 3.2.1.1.) was purchased from Novo Nordisk BioChem. Inc. (Franklinton, NC).

Extraction of β-glucans

The extraction procedure of Wood et al. (1978) was carried out with the following modifications. The extraction procedure is shown in Figure 2.1. Extraction was performed on 200 g batches of barley flour. Enzymes in flour were inactivated by refluxing in 70% ethanol twice (1h each), flour was dried in a fume hood and reground in a Udy mill. For the extraction of refluxed flour and/or for the extraction at higher pH (9 or 10) levels, the water:flour ratio was increased from 10:1 to 12:1 for ease of mixing and separation of phases. The centrifugation force was 17,600 x g for 20 and 25 min for the separation of starch and protein impurities, respectively. Following precipitation with ethanol and centrifugation, gum was air dried at 80°C and pulverized. For the preliminary runs (Tables 2.1 - 2.3), flour was wetted in distilled water for 15 min prior to pH adjustment. One series of refluxed samples underwent Termamyl treatment with enzyme digestion for 1 h at boiling point to eliminate starch impurities.

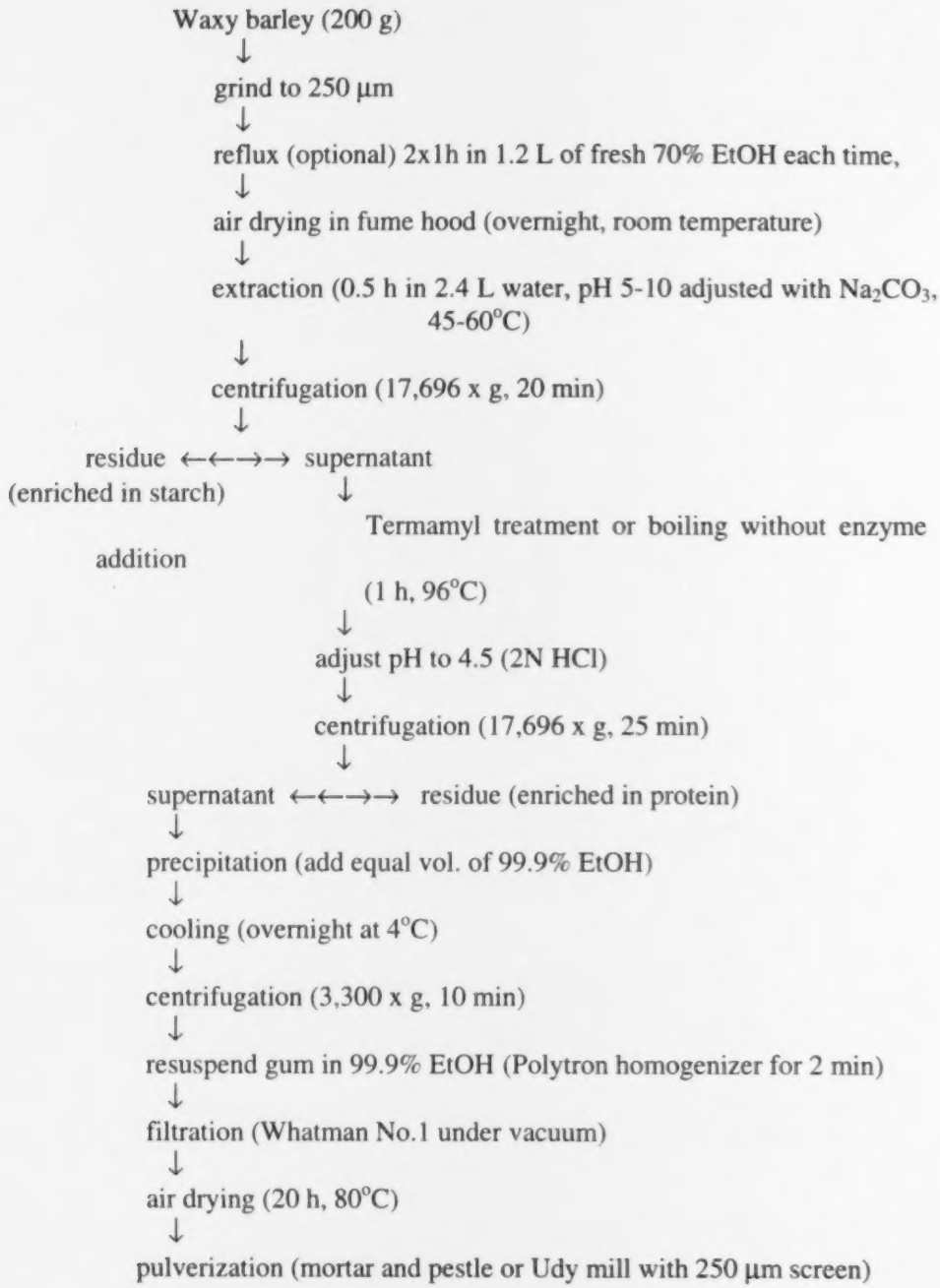

Waxy barley (200 g)
grind to 250 µm
reflux (optional) 2x1h in 1.2 L of fresh 70% EtOH each time,
air drying in fume hood (overnight, room temperature)
extraction (0.5 h in 2.4 L water, pH 5-10 adjusted with Na_2CO_3, 45-60°C)
centrifugation (17,696 x g, 20 min)
residue ←←→→ supernatant
(enriched in starch)
Termamyl treatment or boiling without enzyme addition
(1 h, 96°C)
adjust pH to 4.5 (2N HCl)
centrifugation (17,696 x g, 25 min)
supernatant ←←→→ residue (enriched in protein)
precipitation (add equal vol. of 99.9% EtOH)
cooling (overnight at 4°C)
centrifugation (3,300 x g, 10 min)
resuspend gum in 99.9% EtOH (Polytron homogenizer for 2 min)
filtration (Whatman No.1 under vacuum)
air drying (20 h, 80°C)
pulverization (mortar and pestle or Udy mill with 250 µm screen)

Figure 2.1. Flow chart of β-glucan extraction from waxy barley flour.

Gum samples were coded as follows: the first letter describes the barley cultivar, K for Condor and W for Waxy. The second letter(s) represents the thermal treatment a gum passed through:

R - barley flour was refluxed in 70% ethanol.

T - Termamyl treatment was applied on the extract.

B - no ethanol reflux of flour, extracts were boiled immediately after first centrifugation for 1 h.

P - no ethanol reflux of flour, but immediate addition of 20% Na_2CO_3 to flour, followed by addition of water and adjustment of pH before extraction, no thermal treatment of extract.

The numbers in the sample code represent the pH and temperature of extraction.

Classification of β-glucan gums as low, medium and high viscosity types (LV, MV and HV, respectively) was not precisely defined in the literature. Therefore, gums were classified as follows in this study based on the viscosity of 1% (w/w) gum solutions: LV 1-20 cP, MV 20-100 cP and HV >100 cP. Also, an ultra high viscosity category (UHV, >300 cP) may be used as necessary.

Viscosity measurements

Viscosity measurements were performed on a Haake Rotoviscometer (model RV-3, Gebruder Haake, Berlin, Germany) at lower speeds to approach the shear rate of eating range of 20-50 s^{-1} (Glicksman, 1982). Pseudoplastic behavior as described by the power law model was determined by conducting flow curve or consecutive fixed speed tests. The stability of gums was tested by measuring the

viscosity of the extracts as a function of time with the point prior to final precipitation with ethanol being t=0.

For viscosity measurements gums were prepared in the desired concentration (0.5% or 1% w/w) by dissolving in distilled water, heating to a boil and maintaining the temperature around the boiling point for about 10 min. Then, the solution was cooled and maintained at ~60°C for 1 h on the multiposition hot plate stirrer, cooled down to room temperature on a magnetic stirrer and the concentration was adjusted with distilled water to make up for evaporation losses. R and RT gums were hydrated according to this first method. However, the hydration treatment was modified to minimize the thermal breakdown of β-glucan. B and P gums were only brought to boiling, removed and further stirred at ~77°C for 1 h on the multiposition hot plate stirrer (low power). Then, the solution was cooled on the magnetic stirrer and concentration was adjusted with distilled water.

Chemical analysis

β-Glucan and moisture contents of gum samples were determined according to McCleary and Glennie-Holmes (1985). Starch and pentosans were quantitated by the method of Holm et al. (1986) and Hashimoto et al. (1987), respectively. Proteins were analyzed using a FP-428 Nitrogen Determinator (Leco® Corp., St. Joseph, MI). Ash content was determined according to AACC Method 08-01 (AACC, 1984).

Statistical analysis

Extractions at each condition were performed in two replications and the chemical analysis of each extract were conducted in duplicate. Viscosity measurements were carried out in triplicate for each sample. Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). Multiple comparison of the means was performed by least significant difference (LSD) test at α=0.05 level.

2.3. RESULTS AND DISCUSSION

Yield, composition and viscosity of gums

Preliminary extractions were conducted with Condor and Waxy barley at pH 4.5-9 and 45-60°C for 0.5-2 h. The yield and composition of the products are presented in Tables 2.1 and 2.2 and the viscosity data of the 0.5 and 1% gum solutions are given in Tables 2.1 and 2.3 for Condor and Waxy barley, respectively. Total product yield increased as the pH decreased, however β-glucan purity decreased due to extraction of other components under acidic conditions. The yield of gums extracted at pH 8 was lower than that of gums extracted at pH 4.5-7 range, but their purity was higher (72.0 and 68.5% with and without a protein separation step, respectively) so that the yield of β-glucan was slightly (<10%) lower than that at lower pH levels (Table 2.2). Since our goal is a BBG gum with high viscosity and purity, ultimately to be used as a thickener and stabilizer, extraction at pH 8 was preferable despite lower yields. A longer extraction time substantially increased the yields of both gum and β-glucan. This

Table 2.1. Composition and properties of gum extracted from Condor (K) flour.

Conditions		Yield,	Proteins	β-glucan	Conc. of	Viscosity,
pH-temp.	Time, h	%	%	%	gum, %	cP
7-55	0.5	5.5	6.1	67.2	0.5	2.3
7-55	0.5				1.0	4.9
7-55	0.5				2.0	20.7
5-55	0.5	6.2	5.8	62.2	1.0	2.9
5-60	2.0	7.6	4.1	57.8	1.0	3.1

Table 2.2. Composition of gums extracted from Waxy (W) flour.

Conditions		Yield,	Proteins,	β-glucan,	Yield of
pH - temp.	Time, h	%	%	%	β-glucan, %
4.5 -55	0.5	8.0	3.1	56.9	4.6
5-55	0.5	8.3	5.5	46.1	3.8
5-55	2.0	11.5	3.0	40.8	4.7
5-60	2.0	14.8	1.8	33.1	5.0
5.5-55	0.5	7.5	6.7	58.1	4.4
6-55	0.5	7.8	8.0	55.4	4.3
6-55	1.0	8.1	6.9	57.3	4.6
6-55	2.0	9.4	4.9	56.3	5.3
7-55	0.5	6.9	6.5	65.4	4.5
8-55	0.5	6.3	6.2	68.5	4.5
7-55	0.5 PP[1]	6.6	6.0	65.6	4.3
8-55	0.5 PP	6.0	5.6	72.0	4.1
8-55	1.0 PP	6.3	5.4	67.3	4.2
8-55	2.0 PP	6.7	5.3	65.5	4.4
8.5-55	0.5 PP	6.1	6.4	61.6	4.2
9-45	0.5 PP	5.6	16.2	48.6	2.7

[1] Proteins precipitated as in Fig. 2.1 procedure. The lack of letters indicates that this step was not done.

Table 2.3. Viscosity of some 1% (w/w) gum solutions extracted from Waxy flour at different conditions.

Gum	Extraction time, h	Viscosity[1], cP
W 4.5-55	0.5	7.7
W 5.5-55	0.5	6.4
W 6-55	0.5	6.4
W 7-55 PP[2]	0.5	12.8
W 8-55 PP	0.5	27.9
W 8-55 PP	1.0	28.6
W 9-45 PP	0.5	31.8

[1] Determined as flow curve viscosity.

[2] Proteins precipitated a in Fig 2.1 procedure. The lack of letters indicates that this step was not done.

increase was more pronounced at lower pH which was closer to the optimum activity of β-glucan solubilase, the enzyme that helps the release of β-glucan from the protein matrix of cell walls. At pH 7 and 55°C, the β-glucan yield from waxy barley (4.5%) was higher than that from Condor (3.7%). This is due to the higher β-glucan content of waxy flour compared to that of Condor barley, 7.0 vs. 5.2%. A temperature of 55°C was selected because Temelli (1995) showed that pH 7 and 55°C gave the highest β-glucan recovery from Condor barley among the conditions studied (pH 7-10, 40-55°C). Higher pH enhances the solubilization of proteins (Hurrel and Pinot, 1985). Thus, protein precipitation (PP in Table 2.2) is recommended when pH >7. Even with PP it was impossible to properly separate phases when extraction was carried out at pH 9/55°C.

The viscosity of extracts increased with the pH of extraction (Tables 2.1 and 2.3) and was the highest for the sample extracted at pH 9 in spite of its lower purity. When one sample extracted at pH 8 was left to sit for about 2 h at 4°C prior to centrifugation, the product viscosity dropped from >20 cP to ~12 cP. This preliminary result was an indication that β-glucanase enzymes are extracted together with gum and that further steps have to be undertaken to ensure their inactivation.

Based on the results of the preliminary extractions, it was decided to use waxy barley as a raw material for the further extractions. Immediate pH adjustment with the addition of 20% (w/v) Na_2CO_3 followed by extraction with no additional treatment (P), boiling the extract after starch centrifugation (B), refluxing the flour with ethanol prior to extraction (R) and Termamyl treatment of

extract from refluxed flour (RT) were the treatments studied to minimize the activity of β-glucanase enzymes and maximize viscosity. Literature (Table 1.5) offered a rather wide range of inactivation conditions. Either ethanol or isopropyl alcohol (IPA) were the chosen inactivating solvents. Ethanol was our solvent choice for refluxing since it is used widely in the food industry and does not represent a health risk as it is used in this application. However, ethanol refluxing is expensive requiring special fire and explosion proof equipment and plant construction. It stops native barley enzymes, but develops chemical instability. Thus, developing another method to inactivate enzymes resulting in a gum product of comparable yield, purity and viscosity would be beneficial. Therefore, boiling of the supernatant from the starch separation at extraction pH was investigated as an alternative stabilization method. On the other hand, oat flour is stabilized by heat treatment for Oatrim® production (Jenkins, 1996).

The composition and viscosity of gums prepared using the above treatments are presented in Tables 2.4-2.7. Although P gums had high viscosity (Table 2.4), it was impossible to separate the extract from the protein fraction properly. Consequently, the β-glucan concentration in gums was lower, dropping to 41.7% and protein contamination was high reaching 25.4% at pH 10 and 55°C. Despite increasing centrifugal force to 21,000 x g, protein separation was not improved. Forces higher than 8,000 x g are rarely applied in industrial production, thus this method was excluded as a potential step in scaled up production of BBG gums.

Table 2.4. Composition and viscosity of gums (P) extracted at strong pH.

	Gum	
	W P 9-55	W P 10-55
Yield, %	4.7	5.2
Moisture, %	7.1	6.5
β-Glucan, %	53.7	41.7
Proteins, %	16.4	25.4
Viscosity of 1% (w/w) solution, cP, 16 rpm	150.1	131.1

When barley flour was refluxed (R) (Table 2.5), the viscosity of samples extracted at pH 8 and 9 was more than twice that of samples extracted at pH 5 and 6.Higher pH samples also had higher purity. However, in spite of much higher viscosity the β-glucan content of the gums was substantially lower when compared to samples obtained without reflux (~56 vs. ~ 68%). The reason for this drop was the high degree of starch recovery due to its gelatinization during refluxing. The presence of starch contributes to viscosity. In addition to decreasing β-glucan purity, it may also provoke an instability of β-glucan solutions (Ch. 3). Therefore, minimizing starch presence through its hydrolysis with Termamyl treatment as described in Bhatty (1995) was undertaken.

When boiling (B) of extract was tested as a method for enzyme inactivation (Table 2.6), high viscosity (>100 cP for 1% solution) was achieved for gums extracted at pH 9 and 10. Even though the highest β-glucan content (72.2%) was achieved at pH 8, its viscosity was rather low (56.9 cP) indicating some enzymatic hydrolysis. W B 8.6-55 gum was subjected to different thermal treatments: boiling for 1 h vs. holding at 90°C for 30 min. Although the latter was insufficient to kill active enzymes, the gum obtained after heating at 90°C had 45% higher viscosity. This was another indication that β-glucan is not stable at boiling temperatures. Therefore, boiling should be applied with care.

Termamyl treatment of the extract from refluxed flour (RT) resulted in a substantial improvement in the purity of gums, reaching >72% β-glucan (Table 2.6). The presence of starch was decreased from ~25% in the R gums to <2% in RT gums, while the protein content showed a >60% increase compared to R

Table 2.5. Composition and viscosity of gums extracted from refluxed flour.

Gum	Yield, % as is	Moisture, %	β-glucan, %	Starch, %	Protein, %	Pentosans, %	Viscosity, cP, 0.5% soln, 64 rpm
W R 5-55	7.0	5.4	49.7	23.4	2.0	5.0	8.9
W R 6-55	6.9	5.4	51.3	24.7	2.2	3.9	8.8
W R 7-55	6.1	5.5	54.3	25.6	3.2	4.3	13.3
W R 8-55	7.1	5.4	56.3	24.1	4.4	4.1	20.8
W R 9-55	8.0	4.7	57.5	24.7	4.5	4.6	21.4
W R 10-55	8.9	5.6	51.6	24.2	6.5	5.9	15.7

Table 2.6. Composition and properties of gums extracted from refluxed flour with Termamyl treatment and untreated flour stabilized by boiling.

Gum	Yield, %	Moisture, %	β-glucan, %	Starch, %	Protein, %	Pentosans, %	Ash, %	Viscosity, cP, 0.5% soln, 16 rpm
Gum stabilized by boiling (B) of extract								
W B 8-55	6.0	5.8	72.2	n.d.	2.0	n.d.	1.9	56.9[1]
W B 8.6-55 boiled 90°C	6.5	4.8	54.9	n.d.	5.7 6.2	n.d.	8.8	52.9[1] 76.9[1]
W B 9-55	5.3[b]	6.0	65.2[b]	11.3	5.7	6.2	2.1	25.0[c]
W B 10-55	5.4[ab]	6.5	64.1[b]	11.8	4.9	5.9	1.6	30.5[c]
Gum stabilized by reflux and Termamyl treatment (RT)								
W RT 8-55	5.4[ab]	5.0	75.3[a]	1.6	8.0	6.5	1.9	48.8[a]
W RT 9-55	5.5[ab]	5.3	73.8[a]	1.0	8.8	6.9	2.4	44.3[b]
W RT 10-55	6.1[a]	5.9	72.9[a]	1.1	10.0	6.8	2.1	28.2[d]

[a, b, c, d] Means in the same column with the same letter are not significantly different ($p>0.05$). Only HV samples included in statistical analysis.

n.d. not determined.

[1]Viscosity of 1% (w/w) solutions.

gums. Despite the harsh thermal treatment, viscosity was somewhat higher following RT treatment. Conducting Termamyl treatment at a lower temperature may improve viscosity further since the concentration of β-glucan in the gums was substantially increased. Viscosity of 0.5% gum solutions was reported for R and RT treatments because the viscosity of 1% solutions was beyond the range of the Haake viscometer. Since the major objective of this study was to obtain high viscosity gums, only those HV gums with a high β-glucan content (B pH 9, 10 and RT pH 8, 9, 10) were included in statistical analysis (Table 2.6). The effect of pH on total yield and β-glucan content was not significant ($p>0.05$) within each treatment even though β-glucan content showed a decreasing trend with increasing pH. The β-glucan content of gums obtained with RT treatment was significantly ($p<0.05$) higher than that of B gums. pH effect on viscosity of B gums was significant ($p<0.05$) with higher viscosity for pH 10 than for pH 9 (30.5 vs. 25.0 cP, respectively). Viscosity of W RT 8-55 gum was significantly higher than that of any W RT gum, and viscosity decreased significantly ($p<0.05$) with pH. The recovery of β-glucan was 49.1 and 49.5% for W B 9-55 and W B 10-55 gums, respectively which was lower than that for W RT 8-55 (53.1%) and W RT 9-55 (53.3%) gums. This difference (>3.6%) may have substantial economical impact in a large scale plant. The recovery of β-glucan for W RT 10-55 gum was the highest, 58.5%, but its viscosity was >36% lower compared with W RT 8-55 and W RT 9-55 gums and may be recomended only if high viscosity is not a goal of extraction.

A trial run at pH 10 and a higher temperature of 60°C gave promising results for high yields with somewhat decreased viscosity compared to runs performed at 55°C. Thus, a series of extractions with an initial pH of ~10.2 (in order to maintain pH ~10 during extraction) and temperature of 60°C was performed. However, results (Table 2.7) were not satisfactory. The viscosity of samples was only about half of that obtained at 55°C which may be due to cleavage of (1→3) bonds under high pH conditions. Protein impurities were not as high (4.4-6.8%, usual values are 5-10%), but they were hardly soluble and in the form of flakes when the gum was redissolved. Consequently, pH 10 and 60°C cannot be recommended for the extraction of β-glucan gum.However, multiple extractions carried out at pH10/60°C give an indication of the variability in the data (Table 2.7).

Stability of gums

The stability of the gum products was assessed by determining the change in its viscosity as a function of time. The drop in viscosity over time is an indication of β-glucanase activity which was not possible to detect otherwise since the existing assay techniques (β-glucanase (malt and microbial) assay kit, Megazyme Australia Pty. Ltd, Warriewood (Sidney), NSW, Australia) are not sensitive enough to detect such low levels.

Figure 2.2 depicts extraction of active enzymes together with P gums at pH 9 and 10. The activity of these enzymes was slowed down if the pH was returned to extraction value, but remained high if the pH was maintained at 4.5 following protein separation since 4.5 is close to their optimum pH. This was in

Table 2.7. Composition and viscosity of gums extracted at pH 10 and 60°C from refluxed flour with Termamyl treatment.

Gum	Yield, %	Moisture, %	β-glucan, %	Protein, %	Pentosan, %	Viscosity, cP, 0.5% conc., 32 rpm
W RT 10-60-1	6.5	5.5	66.4	4.8	3.1	6.9
W RT 10-60-2	7.0	5.3	64.6	6.8	3.0	9.8
W RT 10-60-3	7.0	5.4	65.0	4.9	3.9	9.9
W RT 10-60-4	7.1	5.2	66.2	4.4	3.0	12.9
Mean ± σ	6.9 ± 0.28	5.4 ± 0.12	65.6 ± 0.87	5.2 ± 1.09	3.3 ± 0.43	9.9 ± 2.47

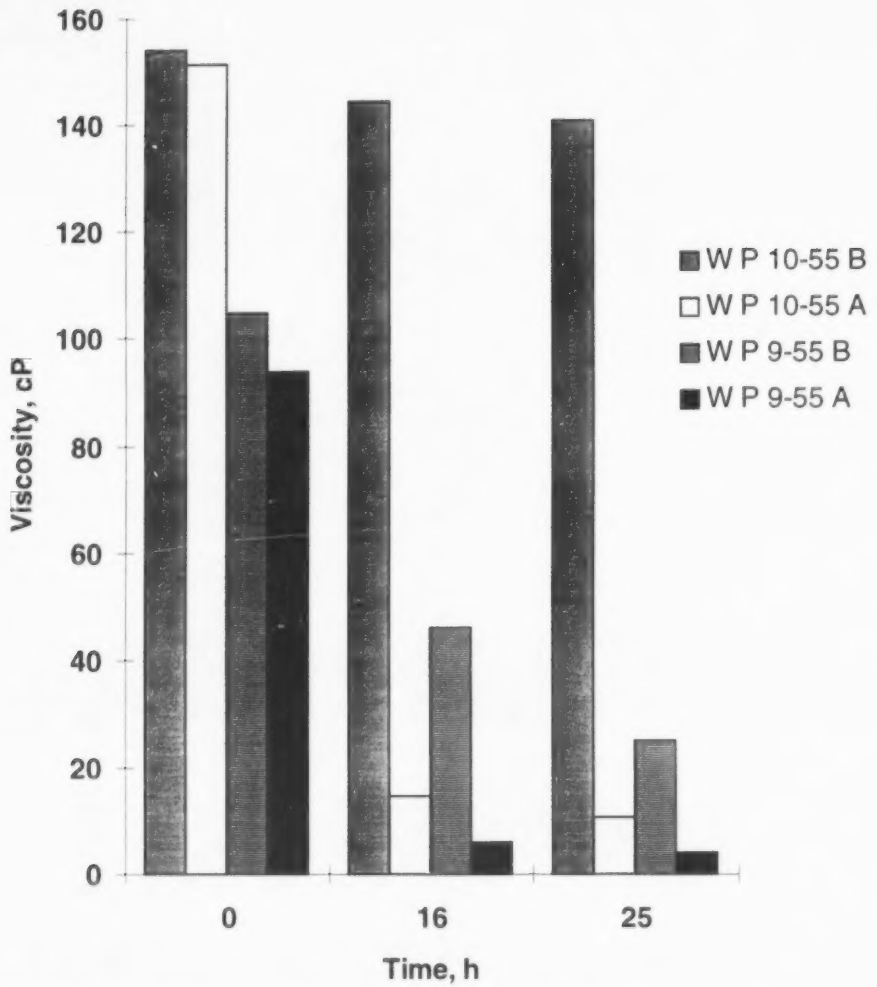


Figure 2.2. Stability of 1% gum solutions of W P 9-55 and W P 10-55 determined at pH 4.5 (A) and pH of extraction (B). Viscosity was measured at 16 rpm.

agreement with the findings of Wood et al. (1978) who also found enzyme activity in BBG gum after extraction at pH 10.

The viscosities of boiled gum solutions are presented in Table 2.8 as a function of time. The first set of runs was not successful resulting in a substantial viscosity loss after 24 h. Further improvements in the boiling step to ensure uniform heating and mixing of foam back into solution resulted in an extract that was stable for 3 d when pH was maintained at 9 following extraction. However, when the extract was kept at pH 4.5 following centrifugation its viscosity dropped slowly but steadily which may be due to microbial contamination. The viscosity of W B 9-55 extract was stable for 19 h. The loss of viscosity in the pH 10 extract was somewhat surprising. However, higher ionic strength and high pH could have led to the cleavage of (1→3) bonds in β-glucan molecules (McCleary and Codd, 1991). Extracts obtained at lower pH of 8 and 8.6 were tested for stability as well, but lost about half of their viscosity in 24 h. Consequently, extracts of pH <9 were precluded from further testing for stability.

When refluxed samples were tested for stability (Table 2.9), it was surprising that pH 7 was more beneficial for the stability of extracts than pH 9. This might well be due to chemical cleavage of polysaccharides which occurs as a result of the action of phenolic compounds (Madacsi et al., 1983). Microbial enzymes, present on the outer layers of the grain, are much more heat resistant and may have survived the inactivation procedure (Wood and Weisz, 1987).

It is noticeable that boiling of extracts obtained from refluxed flour did not improve viscosity (Table 2.9). Even though boiled extracts of W R 7-55 had a

Table 2.8. Stability of boiled gum solutions determined by measuring viscosity with time at 32 rpm.

W B 9-55			W B 10-55		
Time	Viscosity, cP		Time	Viscosity, cP	
	A[1]	B[2]		A[1]	B[2]
0	73.7	76.2	0	71.3	60.8
19 h	74.1	77.4	18 h	29.1	55.6
28 h	68.0	76.9	72 h	19.5	9.2
72 h	62.1	76.1			
144 h[3]	-	64.2			

[1] pH ~ 4.5.
[2] pH returned to ~9.
[3] Solution A was spoiled.

Table 2.9. Stability of refluxed gum solutions determined by measuring viscosity in time at 32 rpm.

Gum	W R 7-55		W R 7-55 - boiled		W R 9-55		W R 9-55 - boiled	
Time	A[1]	B[2]	A	B	A	B	A	B
0	29.6	29.6	40.0	39.2	50.9	50.3	38.2	36.3
2 h	32.0	31.4	38.9	39.2	50.4	49.3	35.5	36.0
16 h	28.9	29.6	36.8	38.7	46.8	44.1	29.9	36.2
24 h	27.4	29.5	38.3	38.9	45.1	42.9	28.6	35.7
120 h[3]	25.8	-	35.8	-	35.2	-	25.6	-

[1] pH ~ 4.5.
[2] pH returned to extraction value.
[3] All B samples were spoiled.

viscosity that was ~30% higher than that for extracts not boiled, they lost 1/3 of their volume and were expected to have at least 70% higher viscosity. Extracts of W R 9-55 had ~25% lower viscosity after boiling in spite of evaporation. Higher extraction pH also resulted in higher viscosity of extracts. Higher pH causes digestion of cell wall proteins releasing longer β-glucan molecules which produce higher viscosity. On the other hand, higher pH also decreases the chain length when a higher temperature treatment is applied.

When samples treated with Termamyl after reflux were studied for enzyme activity, no change in viscosity was detected for 24 h (Fig. 2.3). High pH of 10 and 60°C in combination probably contributed to digestion of proteins (Hurrel and Finot, 1985). In order to test if sterilization might improve stability, an aliquot of the extract W RT 10-60 was sterilized at 121°C for 20 min (Fig. 2.3). A substantial drop in the viscosity of the sterilized sample was surprising. However, such thermal degradation was in agreement with the findings of Gelfand (1996).

Effect of shear on viscosity of gums

HV and LV pure gums (Megazyme, Australia) and W B 9-55 gum were tested for shear sensitivity. The results are presented in Table 2.10. The behavior of LV pure gum resembled Newtonian rather than pseudoplastic behavior. A similar behavior was exhibited by 0.25% solution of W B 9-55 gum, whereas 0.5% and 1% solutions of both W B 9-55 and HV pure gum behaved as pseudoplastic liquids (Fig. 2.4 and 2.5) having a flow behavior index much lower than 1 (Table 2.10). The values of flow behavior index (n) for 1% HV gums are comparable to those reported by Bhatty (1995) for 1% barley and oat gum

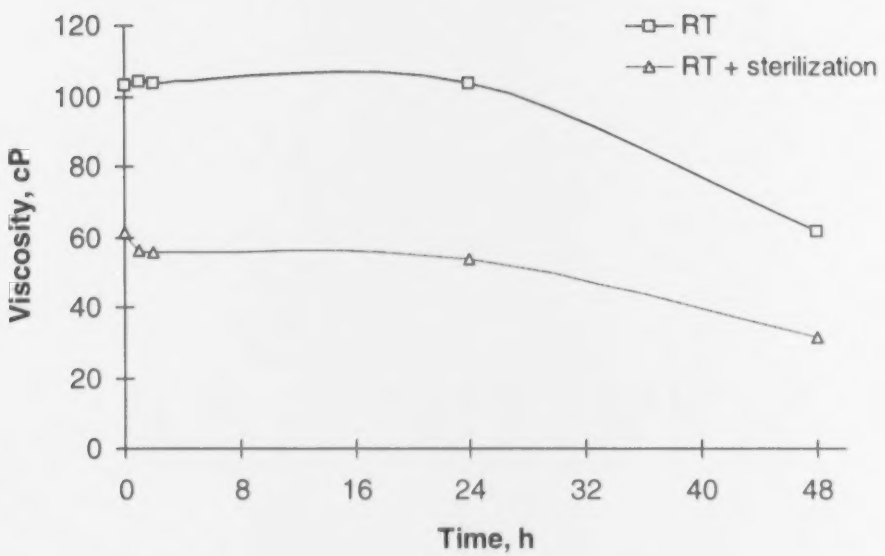


Figure 2.3. Stability of W RT 10-60 extract with and without applied sterilization. Viscosity was measured at 32 rpm.

Table 2.10. Summary of pseudoplastic behavior of HV and LV pure gum and W B 9-55 gum according to Power Law equation.

Gum	Concentration, % (w/w)	Consistency coefficient, c	Flow behavior index, n	R^2
HV pure gum	0.5	0.35	0.904	0.9993
HV pure gum	1.0	4.03	0.803	0.9989
LV pure gum	1.0	0.10	0.999	0.9993
W B 9-55	1.0	2.55	0.860	0.9998
W B 9-55	0.5	0.33	0.885	0.9982
W B 9-55	0.25	0.04	1.002	0.9982

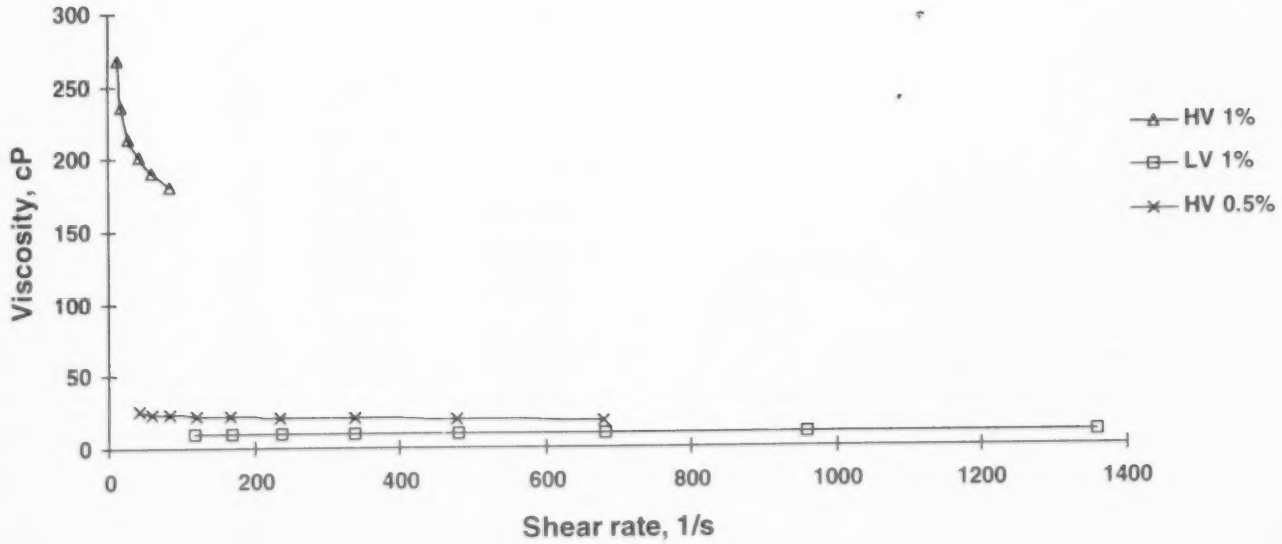


Figure 2.4. Effect of shear rate on viscosity of HV and LV pure gum solutions at different concentration levels.

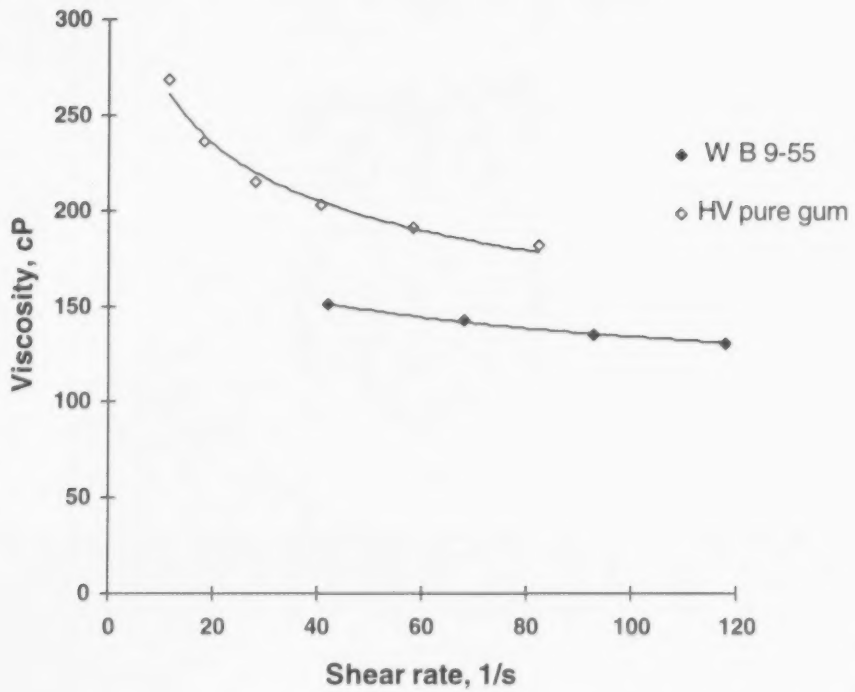


Figure 2.5. Viscosity versus shear rate plot for 1% (w/w) solutions of HV pure gum and W B 9-55.

solutions (0.81-0.89). Consistency coefficient (c) values for W B 9-55 and HV pure gum were higher than those determined by Bhatty (1995) (2.55 and 4 versus 0.096-0.76). Gum solutions with values of n closer to Newtonian behavior (n=1) have a slippery mouthfeel (Szczesniak and Farkas, 1962) and may be used as a fat replacer. Figure 2.5 depicts pseudoplasticity of 1% solutions of HV pure gum and W B 9-55. HV pure gum has a higher viscosity and, consequently, exerts higher pseudoplasticity. Viscosity of W B 9-55 was determined as a flow curve and does not show values close enough to eating range (20-50 s^{-1}). Thus, consecutive fix speed measurements applied for HV pure gum better represent expected mouthfeel and are recommended for future work.

2.4. CONCLUSIONS

The production of high viscosity and stable β-glucan gums from barley requires inactivation of enzymes by either refluxing of flour or thermal treatment of the extract. Gums obtained from refluxed flour do not have a high enough β-glucan purity and should be further purified by Termamyl treatment preferably at temperatures below the boiling point. The maximum β-glucan content of 75.3% was achieved at pH 8 and 55°C with RT treatment with a yield of 5.4%. The viscosity of 0.5% solution of this gum was 48.8 cP. If high viscosity is not the major goal, then LV gums can be extracted without any special treatment and stabilized afterwards. Regardless of the ultimate goal, the production of stable barley β-glucan gum is possible and needs further development. High viscosity BBG gums exhibit pseudoplastic behavior with high values of flow behavior

index indicating the possibility for use as a fat replacer and thickener in food systems.

2.5. REFERENCES

AACC. 1984. Approved methods of the American Association of Cereal Chemists. The Association: St. Paul, MN.

Aastrup, S., and Jorgensen, K.S. 1988. Application of the calcofluor flow injection analysis method for determination of β-glucan in barley, malt, wort and beer. J Am. Soc. Brew. Chem. 46: 76-81.

Bhatty, R.S. 1993. Extraction and enrichment of (1→3)(1→4)-β-D-glucan from barley and oat brans. Cereal Chem. 70(1) : 73-77.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22: 163-170.

Dawkins, N.L., and Nnanna, I.A. 1993. Oat gum and β-glucan extraction from oat bran and rolled oats: temperature and pH effects. J. Food Sci. 58: 562-566.

Dawkins, N.L., and Nnanna, I.A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9(1): 1-7.

Gelfand, J. 1996. Personal communication. Alberta Agriculture, Food and Rural Development, Food Processing Development Center, Leduc, AB.

Glicksman, M. 1982. Functional properties of hydrocolloids. Ch. 3 in *Food Hydrocolloids*. Glicksman, M. (Ed.) CRC Press Inc., Boca Raton, FL. p. 49-93.

Hashimoto, S., Shogren, M.D., and Pomeranz, Y. 1987. Cereal pentosans: their estimation and significance. I. Pentosans in wheat and milled wheat products. Cereal Chem. 64(1): 30-34.

Holm, J., Bjorck, I., Drews. A., and Asp N.-G. 1986. Rapid method for the analysis of starch. Starch 38(7): 224-226.

Hurrel, R.F., and Finot, P.A. 1985. Effects of food processing on protein digestibility and amino acid availability. Ch. 8 in *Digestibility and Amino Acid Availability in Cereals and Oilseeds.* J.W. Finley and D.T. Hopkins (Ed.) p. 233-246. AACC, St. Paul, MN.

Jenkins, R. 1996. Personal communication. Rhone Poulenc Co., Washington, PA.

Madacsi, J.P., Parrish, F.W., and Roberts E.J. 1983. Nonenzymic method for determination of beta-glucan in the presence of starch. J. Am. Soc. Brew.

Chem. 41:161. Cited in Wood, P.J., Weisz, J., Fedec, P. and Burrows, V.D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal. Chem. 66(2) : 97-103.

McCleary, B.V. 1995. Personal communication. Megazyme Australia Pty. Ltd, Warriewood (Sidney), NSW, Australia.

McCleary, B.V., and Codd, R. 1991. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55: 303-312.

McCleary, B.V., and Glennie-Holmes, M. 1985. Enzymic quantification of (1→3,1→4)-β-D-glucan in barley and malt. J. Inst. Brew. 91: 285-295.

Preece, I.A., and Mackenzie, K.G.1952. Non-starchy polysaccharides of cereal grains. II. Distribution of water-soluble gum-like materials in cereals. J Inst. Brew. 58: 353-362.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition Vol. 2, Cary, NC.

Szczesniak, A.S., and Farkas, E. 1962. Objective characterization of the mouthfeel of gum solutions. J. Food Sci. 27: 381-385.

Temelli, F. 1995. Effect of temperature and pH on extraction and functional properties of barley β-glucans. Abstract # 22-5. Presented at the 56th Annual Meeting of IFT, Anaheim, CA. June 3-7.

Wood, P.J., and Weisz, J. 1987. Detection and assay of (1→4)-β-D-glucanase, (1→3)-β-D-glucanase, (1→3)(1→4)-β-D-glucanase and xylanase based on complex formation of substrate with congo red. Cereal. Chem. 64: 8.

Wood, P.J., Paton, D., Siddiqui, I.R. 1977. Determination of β-glucan in oats and barley. Cereal Chem. 54(3): 524-533.

Wood, P.J., Siddiqui, I.R., and Paton, D. 1978. Extraction of high viscosity gums from oats. Cereal Chem. 55(6): 1038-1049.

Wood, P.J., Weisz, J., Fedec, P., and Burrows, V.D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66(2): 97-103.

3. INTERACTIONS OF β-GLUCANS WITH DAIRY COMPONENTS, SALTS AND STARCH[1]

3.1. INTRODUCTION

β-Glucan concentrate should be incorporated into a number of food products for the larger portion of the population to benefit from the health traits of β-glucan. Therefore, an understanding of the β-glucan interactions with pure food components as well as multicomponent food systems such as milk or fruit juice is essential. The studies (Autio et al., 1987; Dawkins and Nnanna, 1995) reported to date have dealt mostly with pure components such as sugar or sodium chloride solutions, some reporting conflicting data.

Dawkins and Nnanna (1995) tested oat gum and found that a small amount of salt (0.1%) enhanced viscosity, while concentrations ≥1% resulted in a large viscosity decrease. They applied a high shear rate of 4,000 s^{-1} for 15 s using a Polytron homogenizer to ensure uniform dispersion of their samples, which might have contributed to the decrease in viscosity. On the contrary, Autio et al. (1987) found no influence of salt on the viscosity of oat gum solutions in the salt concentration range 0-10% (w/w). However, it was not clear whether Autio et al. (1987) dispersed oat gum in hot or cold salt solution and how the gum was hydrated. Although salt is only one of the many components of food systems, its influence on β-glucans may give an indication of how β-glucan may behave in

[1] A version of this chapter is to be submitted to the Journal of Food Science for publication.

more complicated food systems. Therefore, β-glucan - salt interaction needs further investigation.

The behavior of oat β-glucan in sugar solution is established without contradiction. Dawkins and Nnanna (1995) added sucrose to 0.5% oat gum solutions which were previously completely hydrated by heating. They found an increase in viscosity for sucrose concentrations of 20-45% and a pronounced decrease at the 65% sucrose level. Dawkins and Nnanna (1995) concluded that the use of oat gum in food systems at high concentrations (≥0.5%) would possibly warrant hydration prior to addition into the formula. Autio et al. (1987) reported similar findings although they did the opposite and dissolved the oat gum in sugar solutions. Impurities in gums, mainly starch, proteins and pentosans were not normally affected by the presence of sucrose.

Interaction of oat β-glucan gum with other food gums such as xanthan, guar and locust bean gum (LBG) has been studied (Nnanna and Dawkins, 1996). It was found that oat gum has a synergistic effect with LBG and, to a lesser degree, with xanthan gum at a total concentration of 0.5% (w/w). However, the mechanism of this synergy is not well understood. In addition, the behavior of β-glucans in complex food systems such as milk has not been investigated. As well, only oat gum has been used in the above mentioned studies. Whether barley β-glucan concentrate behaves similarly to oat gum needs to be confirmed.

Therefore, the objectives of this study were: (a) to determine the effect of barley β-glucan (BBG) on the rheology of milk and its derivatives such as whey, milk serum and whey protein concentrate (WPC), (b) to examine the stability of

the β-glucan - milk system, and (c) to investigate the effect of different salts and starch on the viscosity of β-glucan solutions.

3.2. MATERIALS AND METHODS

Materials

BBG gums were extracted as described in chapter 2. The starting material was waxy (W) or Condor (K) barley with similar codes applied to depict different extraction conditions and methods as defined in chapter 2. Viscosity tests were carried out with commercial skim milk (Lucerne, Edmonton). Whey was produced by bringing the pH of milk to 4.5, heating to ~40°C, centrifuging at 3300 x g for 10 min and filtering through Whatman No. 1 filter paper. Milk serum was the permeate obtained from ultrafiltration of skim milk through Harp™ hollow fiber membrane (model HF 10-20-PP10, Supelco Inc., Beafonte, PA) with 10,000 MWCO. Sodium caseinate from bovine milk was purchased from Sigma Chemical Co. (St. Louis, MO). Whey protein concentrate (WPC) with commercial name Alacen 878 and protein content of 81.5% was the product of New Zealand Milk Products, Inc. (Santa Rosa, CA). Soluble starch, calcium chloride ($CaCl_2x2H_2O$) and sodium citrate (tribasic, dihydride) were from Fisher Scientific Co. (Fair Lawn, NJ). Pure barley β-glucan gums of low and high viscosity (LV and HV, respectively) with viscosity of 5.9 cSt for LV and 114 cSt for HV (1% w/v solution in Ostwald C-type Viscometer at 30°C) and molecular weight of 137,000 and 327,000 daltons, respectively, were purchased from Megazyme Australia Pty. Ltd (Warriewood (Sidney), NSW, Australia).

Preparation of samples

Gums were added to skim milk, whey, 2.5% sodium caseinate solution and milk serum in the desired concentrations, stirred for about 5 min, then quickly brought to a boil and further stirred for about 1 h at ~75°C. For tests with WPC and different salts, gum solutions were hydrated by the same procedure in distilled water, cooled down to room temperature (22°C), the concentration was adjusted and then salts were added in order to keep the gum:water ratio constant (i.e. 10 g of gum solution + 1% salt). A similar hydration procedure was applied to starch and gum-starch mixtures. The beakers were covered with Parafilm®, slowly stirred for 15 min and allowed to stand for another 15 min prior to rheological measurements.

Rheological measurements

The viscosity of solutions was determined using a Haake Rotovisco Viscometer (model RV-3, Gebruder Haake, Berlin, Germany) at 25.2 ± 0.2°C. Rotational speed was adjusted to achieve shear rate values closest to the actual chewing range of 20-50 s^{-1} (Glicksman, 1982). The concentrations of salts tested were 0.02-0.5% for $CaCl_2$, 0.1-3% for NaCl, 0.05-0.2% for Na-citrate and 1 and 2% for WPC, all expressed as w/w. Viscosity measurements were carried out in triplicate for each sample.

3.3. RESULTS AND DISCUSSION

Interaction with milk and its components

Preliminary runs performed with K 7-55 (LV) gum confirmed that there is a synergistic interaction between β-glucan and skim milk and sodium caseinate resulting in more than doubling of viscosity, while the effect of calcium chloride was negligible (Table 3.1). Therefore, interaction of BBG gum with milk and its derivatives was studied further. Results are shown in Table 3.2 for waxy barley gums obtained at different conditions. Gums mixed with skim milk produced the highest viscosity regardless of the extraction conditions. The effect was the highest for lower concentrations of LV gums. If samples were not boiled, this effect would probably be even larger because of lower thermal degradation (Ch. 2).

When mixed with whey, barley gum had a ~50% and ≥70% increase in viscosity at 0.5% and 1% concentration, respectively. These results indicated that there is either a synergistic effect between the gum and milk components, or a clotting effect which demands a higher dragging force when the hydrocolloid net is full of protein molecules and micelles. While whey behavior could not confirm any supposition, the behavior of gum in milk was more in favor of a synergistic effect, since 0.5% gum concentration produced a thinner net and should have resulted in a lower viscosity increase, which was not the case.

Next, barley gum was mixed with 2.5% sodium caseinate solution (Table 3.2). Although this salt is completely soluble in water and does not have the same

Table 3.1. Interaction of K 7-55 gum with milk, sodium caseinate and calcium chloride.

Gum	Salt concentration, %, (w/w)	Gum concentration, %, (w/w)	Viscosity, cP
Interaction with skim milk			
K 7-55		0.0	2.8
K 7-55		0.5	4.1
K 7-55		1.0	12.7
K 7-55		2.0	57.5
Interaction with Na-Caseinate			
K 7-55	2.5	0.0	1.8
K 7-55	2.5	0.5	3.3
K 7-55	2.5	1.0	5.5
K 7-55	2.5	2.0	15.7
Interaction with $CaCl_2$			
K 7-55	0.00	1.0	4.9
K 7-55	0.01	1.0	5.0
K 7-55	0.02	1.0	5.1
K 7-55	0.05	1.0	5.0
K 7-55	0.10	1.0	5.0
K 7-55	0.50	1.0	5.0

Table 3.2. Interaction of waxy barley gums with dairy components expressed as viscosity of solutions.

Gum	Conc., %	RPM	Viscosity, cP					
			Gum	Skim milk	Whey	Na-Casein.	1% WPC	2% WPC
W B 9-55	0.5	16	19.78	49.00	30.24	26.56	20.12	21.33
W B 9-55	1	16	117.88	221.28[1]	205.15[1]	174.41	143.60	134.14
W RT 9-55	0.5	16	55.42	91.01	81.04	70.13	51.39[2]	50.31[2]
W RT 10-60	0.5	32	9.78	27.45	13.89	13.32[3]	7.85	9.09
		181	7.71					
W RT 10-60	1	32	57.68	107.15	96.52	76.37[3]	81.04	85.56
			71.23[4]					

[1] Out of scale the true value is somewhat higher.
[2] Dissolved together with gum.
[3] Dissolved after hydration of gum.
[4] Sample prepared with a shorter boiling period (removed as soon as boiling started).

molecular size and properties of casein micelles, it showed a 30-35% enhancement in viscosity. This viscosity increase was not as high as that achieved with milk or whey. The addition of sodium caseinate even after the hydration of gum resulted in similar effects.

When 0.5% of W RT 9-55 was dissolved in milk serum, the viscosity was ~20% higher than that of 0.5% solution in water (66 cP vs. 55 cP). A similar trend was observed for 1% W P 9-55 gum where viscosity increased from 166 cP to 198.2 cP after dissolving it in serum. Small amount of lactose as well as the presence of some peptides may have a role in this viscosity increase. When left to stand at 4°C for 2 d, the mixture with W P 9-55 gum was stable, but a small amount of precipitate separated after 7 d. After 1 month, the protein-like precipitate was sharply separated with a clear and viscous supernatant.

WPC was the final component added to barley gum in an effort to explain the interactions of gum with milk (Table 3.2). When WPC was dissolved prior to gum hydration, the viscosity was somewhat restricted for W RT 9-55 gum. Otherwise, WPC addition increased the viscosity of gum solutions, except when 2% of WPC was mixed into W B 9-55 HV solution, the viscosity of 2% WPC solution was lower than that of 1% WPC. A higher WPC concentration probably decreased the hydration of β-glucan gum by competing for water. Depending on its final use, WPC is sometimes produced by a rather harsh thermal treatment so that the native proteins are denatured (Jelen, 1996). As a consequence, they may not behave in the same manner as the whey proteins present in milk.

At this point, the mechanism of what appears to be synergism with milk components could not be explained. In addition, both milk and whey were unstable when mixed with β-glucan gum since they separated into two phases; a whitish precipitate and a misty or whey-like upper phase. The upper phase was still viscous indicating minimal precipitation of gum together with proteins. Throughout this period, the pH of the solution changed from ~6.80 to 6.41, which excludes the possibility of destabilization of casein due to a pH change. A similar phenomenon was observed with both LV and HV pure barley β-glucans (Megazyme, Australia). Thus, it was concluded that precipitation of milk proteins was probably caused by β-glucan and not by the other components present in our extracts. One factor that might play a role in the destabilization of milk is the temperature of heating because the separation was less pronounced when milk was heated only up to 60°C instead of boiling. However, when the gum was prehydrated in distilled water, cooled down to 22°C, mixed with cold milk and allowed to stand at 4°C overnight, precipitation occurred again.

A factor that may contribute to the precipitation of milk proteins is the possibility that β-glucan may influence the ratio between soluble and insoluble calcium and thus diminish the stability of casein micelles. It is noteworthy to point out that there was no visible curdling of casein. When the solution was stirred, it was uniform like milk for up to 2 h, depending on the viscosity of solution, after which it precipitated. If the β-glucan-calcium interaction is in part responsible for the precipitation of milk proteins, then addition of more calcium should help stabilize the system. To test this hypothesis two different solutions were prepared

(5g); 2% (w/w) solution of K 7-55 (LV) and 1% (w/w) solution of W RT 8-55 (HV), and mixed with an equal amount of milk. Different amounts of $CaCl_2$ (2, 5, 10 and 20 mg) were mixed into the gum solution so that the final concentration of $CaCl_2$ after combining with milk was 0.02, 0.05, 0.1 and 0.2%. The result was surprising since precipitation did not occur in 0.02% $CaCl_2$ solution of K 7-55 gum and 0.2% $CaCl_2$ solution of W RT 8-55 gum.

When the W RT 10-60 (MV) gum was mixed with milk at 2 and 3% concentrations, precipitation did not occur after 7 d at 4°C, but phase separation started in the 2% solution after 10 d. This may be explained by casein micelles being entrapped in the hydrocolloid network which prevents them from precipitating out and actually stabilizing the system. Even though additional $CaCl_2$ was necessary to stabilize the system at low concentrations of β-glucan or when LV β-glucan was used, $CaCl_2$ would not be needed at higher concentrations of HV gum. As well, HV β-glucan mixture in milk had a pasty consistency and was slippery to the touch. Thus, new applications of β-glucan in pudding-like products are promising and need to be evaluated.

Interaction with salts

Table 3.3 presents the viscosity of 0.5 and 1% (w/w) waxy barley gum solutions containing different levels of sodium citrate. The effect of sodium citrate was somewhat greater on the gum extracted at harsher conditions (W RT 10-60) where the viscosity increased slightly with sodium citrate concentration. For all other gums viscosity fluctuation was within ±5% range.

A similar pattern was observed for the interaction of gums with calcium

Table 3.3. Viscosity (cP) of waxy barley gum solutions with additional sodium citrate.

Gum	Concentration, %	RPM	Na-Citrate concentration			
			0%	0.05%	0.1%	0.2%
W P 7-55	1	16	40.3	40.6	40.9	41.9
W B 9-55	1	8	311.5	311.1	311.7	312.3
W RT 9-55	0.5	16	52.1	50.6	51.9	51.8
W RT 10-60	0.5	32	10.9	11.4	11.4	11.8

chloride (Table 3.4). The gum (W P 9-55) with the highest protein content (Table 2.4) had the highest variation in viscosity. The effect of $CaCl_2$ on the viscosity of gum solutions was negligible at gum concentrations of 0.5% and slightly higher at 1% level. However, all variations were within ± 5% range.

Compared to the other salts, sodium chloride had the greatest effect (> ±5%) on the viscosity of gums with higher protein content (Table 3.5). Viscosity generally increased with salt addition up to 1% level, followed by a decrease with an increase in salt concentration to 3%. Low NaCl concentrations (<1%) may have solubilized the protein which contributed to increased viscosity, while higher NaCl concentration impaired gum hydration (Dawkins and Nnanna, 1995). In general, W P 7-55 gum showed an increase in viscosity with all types of added salts, but this gum had the lowest viscosity of all the 1% gum solutions tested. Thus, salts may not interfere with its hydration to the same extent.

In order to study the influence of high salt concentration on the hydration of gums, a gum sample was dissolved in 10% NaCl solution or in distilled water followed by salt addition to reach 10% level. Viscosity of 1% gum (W P 10-55) solution was 161.16 cP. When 10% NaCl was added to the hydrated gum solution, the viscosity increased to 162.91 cP. However, when the gum was added to 10% NaCl solution without prior hydration, there was a >20% decrease in viscosity to 124.84 cP. This result indicated that the hydration of gum was limited in the presence of such a high salt concentration.

Table 3.4. Viscosity (cP) of waxy barley gum solutions with additional calcium chloride.

Gum	Conc., %	RPM	Concentration of $CaCl_2$					
			0%	0.02%	0.05%	0.1%	0.2%	0.5%
W P 7-55	1	16	40.3	41.9	41.7	41.2	41.5	41.4
W P 9-55	1	16	157.7	161.9	163.2	152.6	150.0	152.2
W RT 9-55	0.5	16	52.1	51.8	52.1	52.2	52.1	52.1
W RT 10-60	0.5	32	10.9	11.0	10.8	10.9	11.1	10.8

Table 3.5. Viscosity (cP) of waxy barley gum solutions with additional sodium chloride.

Gum	Conc., %	RPM	Concentration of NaCl					
			0%	0.1%	0.2%	0.5%	1%	3%
W P 7-55	1	16	40.3	41.1	40.9	41.0	41.0	41.5
W P 9-55	1	16	151.3	150.4	157.5	160.8	161.2	154.0
W RT 9-55	0.5	16	52.1	51.2	51.0	51.1	51.4	50.5
W RT 10-60	0.5	32	10.9	11.6	12.1	12.1	11.8	11.4

Interaction with starch

Starch solutions of 0.5 and 1%, prepared in distilled water by the same procedure applied for gum hydration, had a viscosity of 4.9 cP and 14.0 cP, respectively. The 0.5 and 1% solutions of W B 9-55 gum had viscosity of 20.1 cP and 137.0 cP, respectively. A mixture of 0.5% starch and 0.5% gum had a viscosity of 50.4 cP. Such a 2.5 fold increase in viscosity represents an additive effect and may be significant in the development of new food products. A similar but less pronounced trend was observed in the viscosity of 0.5% solution of W RT 9-55 gum which increased from ~55 cP to ~97 cP after the addition of 0.5% starch. Both gum mixtures separated into two layers following storage at 4°C for several days. The bottom layer was whitish and probably contained starch, while the top layer was clear with decreased viscosity.

In order to further investigate the stability of starch-β-glucan gum mixtures, gum solutions were prepared similar to those used for the stability test of gum-milk mixtures and mixed with an equal amount of 2% starch solution which was prehydrated in the same manner. Mixes were kept directly at 4°C, or heated up to 80°C first and then left to stand at 4°C. It seems that prehydration slowed down the separation of phases which started after 8 d. Hydrophobicity of the hydrogen bonded regions in amylose and β-glucan chains might be in part responsible for such destabilization of the system. In addition, there is competition between β-glucan and starch for the available water.

3.4. CONCLUSIONS

BBG gum has synergistic interaction with milk components resulting in much higher viscosity than that in distilled water. Solutions with whey, Na-Caseinate, and milk serum also had higher viscosities but to a lesser degree. WPC increases the viscosity of β-glucan solutions probably by decreasing the free water left after the hydration of β-glucan chains. β-Glucan reacts with milk triggering precipitation of proteins. The mechanism of precipitation should be investigated further. Precipitation can be prevented either by the addition of $CaCl_2$ or by an increased concentration of MV or HV gum.

Among the different salts tested, NaCl had the greatest effect on the viscosity of β-glucan solutions, especially when applied in concentrations ≥1%. Interactions with sodium-citrate and calcium-chloride were inside ±5% range probably more due to interfering with hydration and interaction with proteins than with β-glucan itself since β-glucan is a nonionic polymer. This study demonstrated that the order of hydration of gums is far more important than the type or level of salt added. For any future food application with a high concentration of soluble components, barley β-glucan gum should be previously hydrated and then mixed with other ingredients.

Starch mixtures with BBG gum have increased viscosity to an extent that may be considered as an additional effect. These mixtures are prone to instability with starch precipitation. The mechanism of instability cannot be completely elucidated at this stage.

3.5. REFERENCES

Autio, K., Myllymaki,O., and Malkki, Y., 1987. Flow properties of solutions of oat β-glucans. J. Food Sci. 52(5): 1364-1366.

Dawkins, N.L. and Nnanna, I.A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9(1): 1-7.

Glicksman, M. 1982. Functional properties of hydrocolloids. Ch. 3 in *Food Hydrocolloids*. Glicksman, M. (Ed.) CRC Press Inc., Boca Raton, FL. p. 49-93.

Jelen, P. 1996. Personal communication. Department of Agricultural Food and Nutritional Science. University of Alberta, Edmonton, AB.

Nnanna, I.A., and Dawkins, N.L. 1996. Adsorption isotherm and effect of gum blends on viscosity and microstructure of oat-gum. J. Food Sci. 61(1): 121-126.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition Vol. 2, Cary, NC.

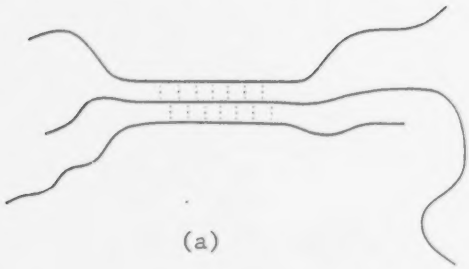
4. GELATION OF BARLEY β-GLUCAN CONCENTRATE[1]

4.1. INTRODUCTION

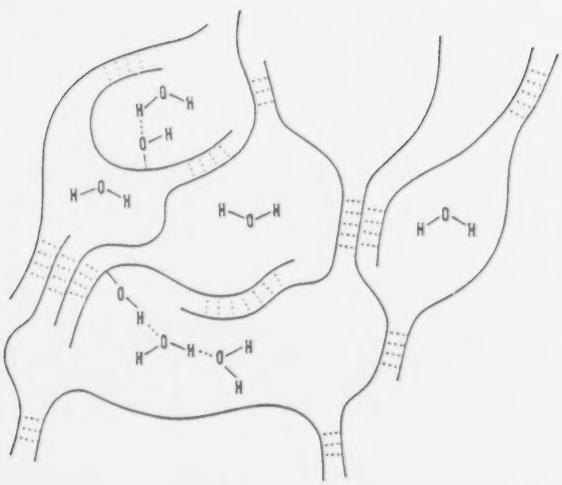
Gelation is the association or cross linking of long polymer chains to form a three dimensional continuous network which traps and immobilizes the liquid within it to form a firm rigid structure that is resistant to flow under pressure (Glicksman, 1982). Gel structure is shown in Figure 4.1. Not all hydrocolloids can form a gel and those that have such ability are agar, carrageenan, alginate, furcellaran, gelatin, pectin and starch. All of them, except starch, are charged molecules and usually need special conditions to form a gel such as the presence of sugars, specific ions (i.e. Ca^{++} for gelation of alginate) and/or specific pH.

The starch macromolecule is not charged. It is composed entirely of glucose units connected by (1→4)-α-glucopyranosyl bonds. Starch has two forms. The straight macromolecule form with no or very slight branching is called amylose, while amylopectin is the highly branched form. Such differences in the molecular structure of amylose and amylopectin impart different properties. Amylopectin does not form a gel in the normal working range concentrations, while amylose does (Glicksman, 1969). Amylose is somewhat similar to β-glucan in structure since both are linear chains of glucose. However, the linkages between the glucose units are different since they are (1→4)-α-linkages in amylose and mixed (1→4) and (1→3)-β-linkages in β-glucan. On the other hand, cellulose has only (1→4)-β-linkages in the molecule, giving it different properties.

[1] A version of this chapter is to be submitted to the Food Hydrocolloids.



(a)



(b)

Figure 4.1. Schemes of intermolecular hydrogen bonding of polysaccharide molecules among chains and in a gel with entrapped water (Hodge and Osman, 1976).

Cellulose chains can bind closer together with hydrogen bonds than starch making it insoluble in water. Such tightly bound regions are crystalline in nature (Glicksman, 1969). Bonds in crystalline regions are so firm that the whole region is devoid of water and becomes hydrophobic in nature.

Since the structure of β-glucan resembles that of amylose and cellulose with regard to their monomer units and linkages, it may be expected that β-glucan can exhibit behavior similar to them. β-Glucan is a straight chain molecule like amylose which is soluble in water. However, it also has cellulosic (1→4) regions which are technicaly insoluble (Table 1.3, MacGregor and Fincher, 1993) and may exhibit attractions to form the knots of a gel network (Figure 4.1 b). There is quite a high probability that β-glucan may set into a gel structure similar to amylose (Figure 4.2). The literature lacks information on the gelation of (1→3)(1→4)-β-D-glucan. Doublier and Wood (1995) observed that partially hydrolyzed oat gum exhibits gel-like behavior. It was the only report regarding the gelation of β-glucan.

The gelation behavior of starch has been studied extensively (Christianson et al., 1985, 1986). In general, the Instron Universal Testing Machine has been used to describe the stress-strain behavior of gels. Bagley et al. (1985) found that the lubrication of platens with paraffin or silicone oil produced reproducible results with 10% gelatin gels. Cross-head speed had no influence on material response for testing times of ≤3 min. Agreement of all stress versus strain data was within ±7% regardless of sample height or deformation rate. The platens in this work were coated with teflon, but Christianson et al. (1985) used Saran®

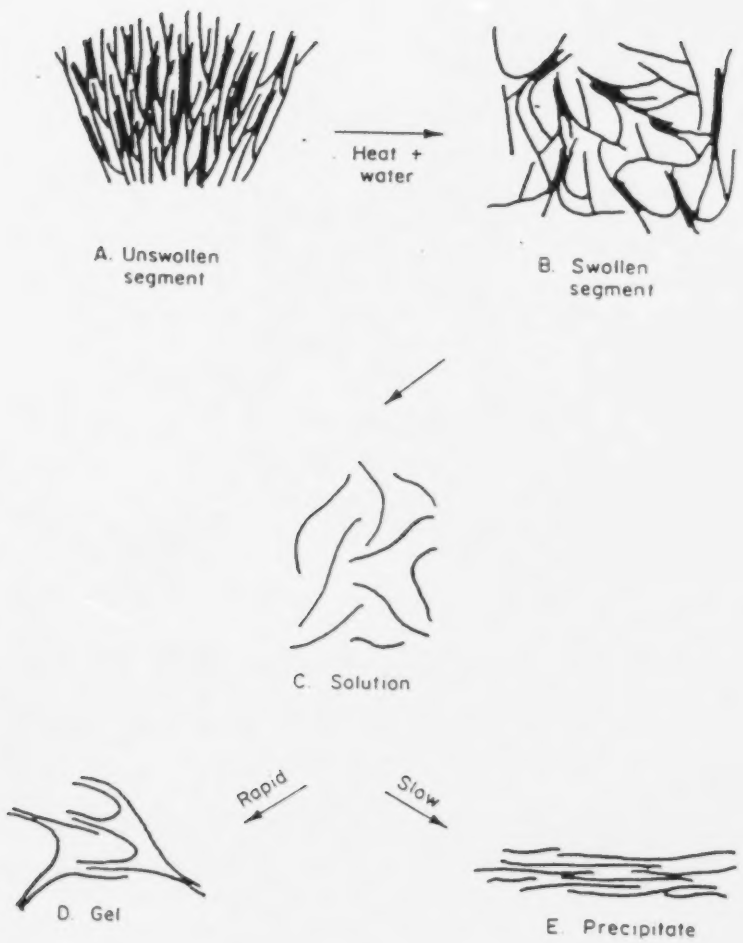


Figure 4.2. Swelling and solubility behavior of amylose (Glicksman, 1969).

covered Lucite® blocks for the testing of lubricated and bonded 10% starch gels prepared by heating at 80 and 94°C for 30 min and obtained satisfactory results. For bonded samples, surfaces were bonded to aluminum foil with cyano-acrylate ester adhesive before testing. Stress versus strain data were dependent on the sample height, so that an additional correction calculation was needed to bring data into agreement with lubricated samples. Compression tests were carried out to 50% deformation using 50 kg load cell at 0.5 cm/min crosshead speed (Christianson et al., 1985). Similar tests were performed by Christianson et al. (1986) when they examined wheat, rice and corn starch gels heated to different internal temperatures. Testing methodologies as described above which were developed for other polysaccharide gels may be appropriate for testing of β-glucan gels, if in fact they do form gels.

Since this thesis represents really pioneering work in the field of β-glucan gelation, the major objectives were to investigate if β-glucan forms a gel at all and to determine the minimal concentration level and conditions required for gelation. With successful gelation additional specific objectives were: (a) to establish a procedure for testing of gel behavior using Instron, (b) to determine the effect of β-glucan concentration on gel behavior, and (c) to study the effect of heating temperature of β-glucan solution on gelation.

4.2. MATERIALS AND METHODS

Materials

β-Glucan concentrates used for the preparation of gels were extracted from non waxy Condor (K) and waxy (W) barley varieties as described in chapter 2. The types of β-glucan concentrates used were coded similarly to those in chapter 2. The duration of extraction was 0.5 h for all gums. Pure barley β-glucan gums of low (LV) and high viscosity (HV) with viscosities of 5.9 cSt for LV and 114 cSt for HV (1% w/v solution in Ostwald C-type Viscometer at 30°C) and molecular weights of 137,000 and 327,000 daltons, respectively, were purchased from Megazyme Australia Pty. Ltd (Warriewood (Sidney), NSW, Australia). The paraffin mineral oil was purchased from Fisher Scientific Co. (Fair Lawn, NJ).

Gel preparation

Barley β-glucan (BBG) gum was dispersed in distilled water in the desired concentration (w/w, as is basis) and stirred for 5 min on a magnetic stirrer to wet the particles. Then the samples were quickly brought to a boil on a multiposition hot plate stirrer (Cole Parmer Instrument Co., Chicago, IL), removed from heater and continued to stir for 1 h at ~77°C on the multiposition hot plate stirrer (minimal power), after which samples were slowly cooled down to room temperature (22°C) with slow stirring. The concentration was adjusted and cool solutions were poured into plastic molds (20 mm x 40.38 mm ID) and held at 22°C for 24 h. Top and bottom of the mold were made of Acryilite® sheets and the whole mold was lubricated with mineral oil. The effect of the level of heating was

studied by heating a 5% solution to 80°C instead of boiling and processing the remaining steps similarly to the other samples.

Preliminary tests to determine whether BBG gums can form a gel were carried out in beakers without pouring into molds. Beakers with prepared samples were covered with Parafilm® and allowed to sit at 22°C or ~4°C.

Mechanical testing of gels

Compressional tests were carried out using an Instron Universal Testing Instrument (model 4201, Instron Corp., Canton, MA) with a 5 kg load cell at a cross head speed of 5 mm/min. Platens were covered with Saran® wrap and profusely lubricated with mineral oil. Instron machine was controlled and data were collected and processed with Instron Series IX software. All gels of each gum at each concentration were prepared and tested in duplicate. Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). Multiple comparison of the means was carried out by least significant difference (LSD) test at α=0.05 level.

4.3. RESULTS AND DISCUSSION

Different concentrations of gums of low, medium and high viscosity (LV, MV and HV, respectively) were tested to determine if they can form a gel. Gum W RT 10-60 (HV) produced from refluxed flour and purified by Termamyl treatment did not gel at concentration levels of 2, 3 and 4% (w/w) after 24 h at 22°C. It was impossible to stir at higher concentrations. Further storage at 4°C did

not help gelation. As well, two other gums, W R 10-55 (HV) from refluxed flour without Termamyl purification and W 7-55 (MV) extracted from non-refluxed barley flour did not gel at 4°C at 2-4% concentration levels.

A similar behavior was observed for the K 7-55 (LV, no reflux) gum at 4% concentration. However, when the concentration was increased to 5% this gum gelled after 3 days at 4°C. A sample of this gum which was heated up to 80°C instead of boiling looked apparently thicker and the gel was firmer to the touch.

The first series of tests demonstrated that a 5% concentration is the threshold for the formation of a gel. Thus, this concentration was used for the next series of tests where W 7-55 (MV) and W 8-55 (MV) gums gelled after 5 days at 4°C. Gum W 8-55 was apparently softer to the touch.

Doublier and Wood (1995) observed that gel-like behavior of oat gums may be obstructed by the high viscosity of gums. In addition, Christianson et al. (1985; 1986) carried out compression tests on starch gels after allowing them to set at 22°C for 24 h. Therefore, the higher viscosity of barley gums held at 4°C may have had a negative influence on gelation. Indeed, when 5% solutions of K 7-55, W 7-55, W 6-55 (LV) and W 8-55 were allowed to set at 22°C instead at 4°C, the setting time was substantially shortened. β-Glucan content and viscosity of these gums are presented in Table 4.1. K 7-55 gum appeared almost gelled after 10 h and was completely gelled after 24 h. Even though W 7-55 gum did not gel in 24 h, it gelled after ~44 h. W 6-55 gum appeared like a soft gel after 48 h despite the fact that it was expected to perform better because of its low viscosity. Lower β-glucan concentration of this gum (Table 4.1) may explain this behavior.

Table 4.1. β-Glucan composition of gels made of different gums.

Gum	Gum conc., %	% β-glucan in gum	% β-glucan in gel	Viscosity of 1% gum solution[1], cP
K 7-55	5.0	67.2	3.36	4.9
K 7-55	5.5	67.2	3.70	4.9
K 7-55	6.0	67.2	4.03	4.9
LV-pure	5.0	90.0	4.50	10.8
W 6-55	5.0	55.4	2.77	6.4
W 7-55	5.0	65.4	3.27	12.8
W 8-55	5.0	72.0	3.60	27.9

[1] Viscosity measured as flow curve viscosity.

The gum W 8-55, which was very difficult to stir, did not gel after 3 days at 22°C but became thicker.

Based on the above preliminary tests it was concluded that: (a) 5% is the minimal concentration for gelation, (b) gum should have a low viscosity <15 cP in order for the gel to set within 24 h and enable handling, and (c) the gum must have a high β-glucan content, >70% (dry matter basis).

As a result, the choice of gum for further experiments was K 7-55 where 4, 5, 6 and 7% solutions of this gum were prepared and allowed to set at 22°C. After 4 h, the 7% solution almost set a gel and 6% solution was very thick, while 4 and 5% solutions were fairly fluid. However, all solutions gelled after 24 h. Even the 4% sample appeared as a very weak gel. It was noticeable that 6% and especially 7% gels retained a lot of air bubbles on top, but were much firmer to touch when compared to 5% gel. Consequently, it was decided to conduct Instron compression tests on the 5, 5.5 and 6% (w/w) gels of K 7-55 gum (as is basis).

Instron compression test

Gels were compressed and resistance was measured until break occurred. During such compression tests, load (kg) and displacement (mm) are the parameters recorded by the Instron machine. These data are used to calculate stress and strain data. Stress (σ, kg/mm^2) is expressed by the equation

$$\sigma = \frac{F}{\pi R^2}$$

where F is the total force (or load) applied over the gels' top surface (πR^2). Strain (S, mm/mm) is calculated as

$$S = \frac{\Delta h}{h_0}$$

where Δh is the change in the height of a sample during compression and h_0 is the initial height (20 mm). A factor that contributes to the variation in the strain data is the difficulty in the bringing of the upper platen manually to exactly the same position for every sample.

Table 4.2 presents the maximal load and displacement at the break point for K 7-55 gels of different gum concentrations. The variation in the load data was <4.6% for all the samples except 5.5% gel which had a relatively high variation of ±7.6% in the maximal load at breaking point. Unfortunately, our samples were not completely free of air bubbles, although they were not visible while pouring into the molds. Those bubbles at the edges could contribute to small variation at breaking point. For further work on gelation, degassing of samples may alleviate this problem.

Figure 4.3 shows stress vs. strain plot for gels of K 7-55 gum at 5, 5.5 and 6% concentration. It is striking that only a 10% shift in concentration from 5 to 5.5% produced almost a three times firmer gel, while a further increase in concentration to 6% resulted in only a 35% increase in gel firmness. Significantly higher ($p<0.05$) loads were required to break the gel with increasing gum concentration (Table 4.2). As well, there was a significant ($p<0.05$) increase in displacement at break point with an increase in concentration from 5 to 5.5%. However, displacement levels for 5.5 and 6% gels were similar ($p>0.05$) (Table 4.2).

Table 4.2. Comparison of gel strengths for gum K 7-55, commercial low viscosity (LV) pure gum and cornstarch gel.

Gum	Gum concen., %	Heating temp., °C	Load, kg	Displacement, mm
K 7-55	5	100	0.353^{ab}	6.39^{b}
K 7-55	5	80	0.315^{a}	5.94^{a}
K 7-55	5.5	100	0.921^{d}	6.81^{c}
K 7-55	6	100	1.247^{e}	6.75^{c}
Corn starch	6	100	0.376^{b}	10.37^{d}
LV pure	5	100	0.642^{c}	6.00^{a}

[a, b, c, d] Means in the same column with the same letter are not significantly different ($p>0.05$).

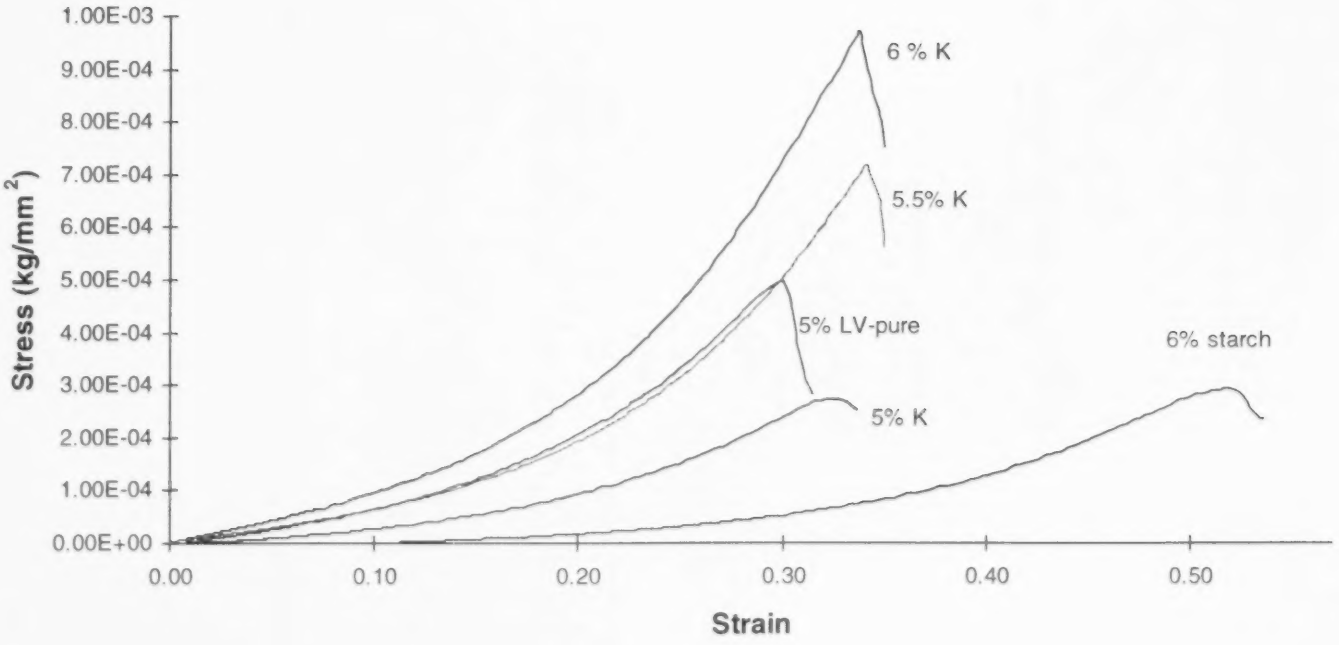


Figure 4.3. Stress - strain plot for different concentration of K 7-55 gels, LV pure commercial barley gum gel and 6% corn starch gel prepared by heating at boiling point.

Heating to 80°C instead of boiling the gum solution produced only slightly ($p>0.05$) weaker gels (Fig. 4.4). The solution heated to 80°C had a higher viscosity and thus required a longer setting time. This delay in setting time could be the reason for the weakness of gels at 24 h.

The gelation behavior of our barley β-glucan concentrates was compared to that of commercial LV and HV β-glucan samples of high purity. However, it was hard to work with HV high purity barley β-glucan. Commercial HV β-glucan, which also had a skinny layer formed on the top, was too thick to stir and maximal concentration achieved was 3.85%. Such behavior was expected based on earlier experience with our HV barley gums. On the other hand, gels of commercial LV gum stuck to the molds in spite of lubrication and were very brittle if the solution was warm as poured into molds. Cooling the solution prior to pouring into molds alleviated this problem. The results are presented in Table 4.2 and Figure 4.1. Gels of 5% pure LV commercial gum was significantly ($p<0.05$) stronger than 5% K 7-55 gels and break occurred at significantly ($p<0.05$) lower displacement (Table 4.2). If the β-glucan content of the gels is compared (Table 4.1), pure LV gum gel had the highest β-glucan content (4.5%) and thus was expected to be the strongest. However, in spite of the highest β-glucan content of the LV-pure gum gel, its strength was significantly ($p<0.05$) lower than 5.5 and 6% gels of K 7-55 gum. Impurities present in K 7-55 gum may also play an important role in the gel structure. Nnanna and Dawkins (1996) recorded that the mixing of oat β-glucan with locust bean gum at an overall concentration of 0.5% resulted in synergistic effect on viscosity. They hypothesized that a mannose backbone devoid of

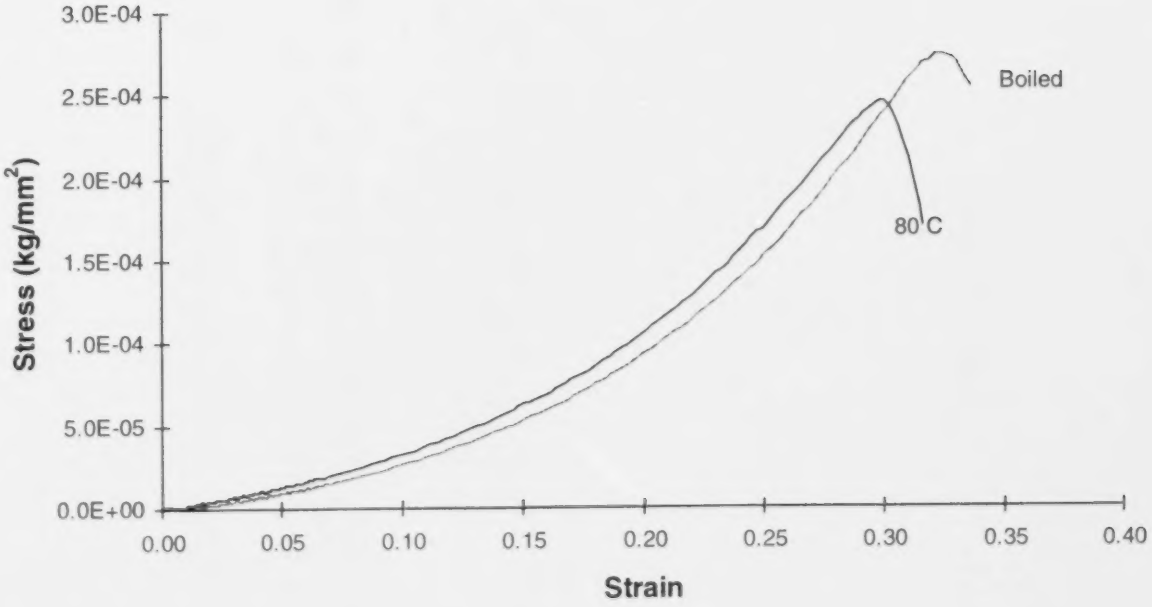


Figure 4.4. Stress- strain plot for K 7-55 gum prepared by heating at 80°C or at boiling point.

galactose units is responsible for this effect. It might be possible that barley β-glucan chains in these gels interact with similar sites on other polymers (proteins, pentosans and starch) producing additional interlinking and a firmer network. These impurities are less hydrated than β-glucan and may link even more readily with β-glucan chains than β-glucan chains link among themselves.

The stress-strain behavior of 6% cornstarch gel is presented in Figure 4.3 for comparison purposes. The cornstarch gel at 6% concentration exhibited different behavior. The cornstarch solution was paste-like and much harder to pour into molds, while β-glucan solutions were more fluid and easier to handle, especially at lower temperatures. When the gel was released from the mold, it sagged immediately losing almost 2 mm in height. Apparently, it was much more elastic, springing back when pressed. While β-glucan gel was more brittle with wedge-shaped cracks after breaking, corn starch gel looked more like an amorphous mass. Strength of corn starch gel was 3.3 times lower than that of the β-glucan gel of similar concentration. This behavior is expected since the gelling compound in corn starch is amylose which is present at a level of 20-28% in cornstarch (Glicksman, 1969). The remaining portion is amylopectin which swells and fills the spaces among amylose network increasing gel strength. The pure amylose concentration of ~1.5% (1/4 of 6%) in the gel mixture is probably not enough to form a strong continuous network by itself.

4.4. CONCLUSIONS

This study established the fact that low viscosity barley β-glucan gums can form a gel at concentration levels ≥5%. Purity and viscosity of the starting gum affect gelation behavior. Gum concentration has a significant ($p<0.05$) effect on gel strength resulting in very large differences in gel strength especially in a rather narrow range of 5-5.5%. Higher gum concentrations (>6%) give short setting times, but also may contain air bubbles which interfere with gel homogeneity and reproducibility of results. Heating the gum solution up to 80°C instead of boiling did not affect ($p>0.05$) the gel strength. Barley β-glucan gum shows potential for the production of food and pharmaceutical gels, but requires further investigation.

4.5. REFERENCES

Bagley, E.B., Wolf, W.J., and Christianson, D.D. 1985. Effect of sample dimensions, lubrication and deformation rate on uniaxial compression of gelatin gels. Rheol. Acta 24: 265-271.

Christianson, D.D., Casiraghi, E.M., and Bagley, E.B. 1985. Uniaxial compression of bonded and lubricated gels. J. Rheol. 29(6): 671-684.

Christianson, D.D., Casiraghi, E.M., and Bagley, E.B. 1986. Deformation and fracture of wheat, corn and rice starch gels in lubricated and bonded uniaxial compression. Carbohydrate Polymers 6: 335-348.

Doublier, J.L., and Wood, P.J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats. Cereal Chem. 72(4): 335-340.

Glicksman, M. 1969. *Gum Technology in the Food Industry.* Academic Press, New York, NY.

Glicksman, M. 1982. Functional properties of hydrocolloids. Ch. 3 in *Food Hydrocolloids*. Glicksman, M. (Ed.) CRC Press Inc., Boca Raton, FL. p. 49-93.

Hodge, I.E., and Osman, E.M. 1976. Carbohydrates. In *Principles of Food Science; Part I., Food Chemistry.* Fennema, O.K. (Ed.), Marcel Dekker Inc., New York, NY.

MacGregor, A.W., and Fincher, G.B. 1993. Carbohydrates of the barley grain. Ch. 3 in *Barley: chemistry and technology.* A.W. MacGregor and R.S. Bhatty (Ed.) p. 73-130. AACC, St. Paul, Minnesota.

Nnanna, I.A., and Dawkins, N.L. 1996. Adsorption isotherm and effect of gum blends on viscosity and microstructure of oat-gum. J. Food Sci. 61(1): 121-126.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition Vol. 2, Cary, NC.

5. CONCLUSIONS AND RECOMMENDATIONS

β-Glucan has been reported to have numerous health benefits. Such reports, together with the nutrition recommendation to increase fiber intake (Health and Welfare Canada, 1990), have generated interest to isolate β-glucans from barley and oats with the ultimate goal of incorporating β-glucan concentrates into food products. The majority of studies have focused on oats which recently led to the commercialization of β-glucan extraction from oats (Canamino Co., Saskatoon, SK).

Barley, which does not have a rancidity problem like oats and performs well in one-step extraction, has a higher β-glucan content than oats, especially waxy hulless varieties. The distribution of β-glucan in the barley kernel is dependent on the cultivar and there are cultivars with higher β-glucan content in the outer layers. There are also barley cultivars which can compete with oats in terms of extract viscosity (Bhatty, 1995). As a result, barley may be quite an economical source of β-glucan when complete fractionation is applied to recover proteins and starch. However, characteristics and potential utilization of such fractions need to be explored.

Two major problems associated with extraction of BBG are the stability and viscosity of the gum product. BBG was extracted from waxy barley at 55°C and pH 8-10using four different treatments: direct extraction with no additional treatment (P), boiling of extract (B), ethanol refluxing of flour (R) and Termamyl treatment of extract from refluxed flour (RT). Stability can be achieved either by the ethanol refluxing of flour or boiling of extract at high pH ≥9. Refluxing of

flour produces high viscosity gum. However, the β-glucan purity of this gum is substantially lowered due to high recovery of starch (20-25% in gum, w/w) gelatinized during refluxing. Thus, further purification with heat stable α-amylase (Termamyl) is necessary. Special attention must be paid during such a purification step since extensive heating over 80°C reduces viscosity. For extracts from refluxed flour, the highest purity (57.5%) and viscosity (21.4 cP, 0.5% soln, w/w) were achieved at pH 9. Too high pH ≥10 can cause a drop in viscosity (<16 cP, 0.5% soln, w/w) and chemical instability.

Boiling of extract produced at pH 9 resulted in a HV stable gum. Relatively high starch impurities may destabilize gum solution. Termamyl treatment can be carried out in addition to boiling, resulting in higher β-glucan content (>70%, as is basis) and higher gum viscosity. A maximum β-glucan content of 75.3% and maximal viscosity of 48.8 cP (0.5% soln, w/w) were achieved at pH 8 with refluxed flour and Termamyl treatment. Extraction at milder conditions pH 7-8.5 without any additional heat treatment results in LV or MV gums. Such gums may find applications in non-liquid food products such as patties, sausages etc. where viscosity stability is not a concern, as a source of soluble fiber, water binder and as a fat replacer (MV gum).

BBG gum exhibits synergistic interaction with dairy components in milk, whey and sodium caseinate at levels of 0.5-1% resulting in a dramatic increase in viscosity ranging from ≥27% (with sodium caseinate) to 250% (1% LV gum in milk). In addition, β-glucan caused sedimentation of milk proteins without visible clotting. Some of the mechanisms by which BBG gum may destabilize casein

micelles are: affecting the equilibrium between soluble and insoluble calcium (Parry, 1974), adsorption of proteins on polymer chains causing the instability of the entire system (Parry, 1974), or small amount (as little as 0.2%, w/w) of proteins present in a gum may trigger the precipitation (Ozimek, 1996). The effect of cetain factors on this phenomenon was investigated by applying different heat treatments of gum-milk mixtures, addition of $CaCl_2$ and application of gums of different purity. Sedimentation of proteins occurred regardless of the degree of heating, but the mixture of cold milk and prehydrated gum precipitated slower overnight at 4°C. The addition of $CaCl_2$ in amounts of 0.02% to LV and 0.2% to HV prehydrated gum mixtures with cold milk stabilized the mixtures for 7 d. When mixed with very pure LV and HV gums (99% purity, Megazyme, Australia), milk precipitated after heating. These pure gums contained small (<0.5%), but significant amount of proteins from the standpoint of stability. A better understanding of the exact mechanism of this phenomenon requires further investigation.

The addition of $CaCl_2$ to milk may stabilize milk - gum mixtures, but the amount must be determined experimentally for each batch depending on its viscosity since a surplus of calcium also can destabilize milk (Parry, 1974). On the other hand, this property of β-glucan to precipitate milk proteins in conjunction with the use of heat and centrifugal force may be used for the concentration of milk protein fractions. Such a protein concentrate containing small amounts of β-glucan may find use in products such as cheese, cheese spreads or dips. The presence of β-glucan represents a value-added product with nutritional benefits.

Higher viscosity of milk - β-glucan mixtures at lower temperatures is desirable in milk-shake or frozen sherbet type products. Application of MV and HV gums at concentrations ≥1% can also stabilize milk turning it into pudding-like products with thick consistency.

Addition of some salts (NaCl, $CaCl_2$, sodium-citrate) at normal usage levels as a food additive resulted in a small (<5%) increase in viscosity except with NaCl where the viscosity increase was larger (>10%). Keeping in mind the destabilization of milk as well, it seems that β-glucan may interfere with the absorption of minerals (Cave et al., 1990). This is a drawback from the nutritional point of view, but it may be a useful trait in pharmaceutical applications if β-glucan may be employed as a special carrier for minerals releasing them in colon where it is degraded by bacteria.

Interaction of β-glucan with starch is of additional type. The viscosity of starch - gum mixtures increases significantly, but is still lower than that of BBG gum applied in the same total concentration alone. Starch - β-glucan mixtures were unstable and behaved similar to mixtures of β-glucan with milk. The 2% solution of BBG-starch mixture (1% each) separated into phases at 4°C after 6 days where the lower starchy layer was cloudy, while the clear upper layer was still thick due to β-glucan presence. This phenomenon is probably the result of starch retrogradation and it may be avoided with the use of waxy starch which does not retrograde.

β-Glucan can form a gel only if it is of the LV type. Otherwise, extensive hydration of HV gums prevents β-glucan chains from coming into close contact and forming a gel network. As well, HV gums are physically impossible to process at ≥5% concentration levels which is necessary for gel setting. MV gums can form a gel, but they need a few days of setting time. A LV gum used for gelation should have a purity of at least 70% β-glucan (dry matter basis). While a cornstarch solution (6% conc.) is rather pasty and inconvenient for handling, a LV β-glucan solution in the same concentration is more fluid and easier to pour (or pump). Investigation of the stress-strain behavior of the gels using an Instron showed that the β-glucan gel was more than three times firmer than the corn starch gel. In addition, β-glucan gel was more brittle, breaking faster under load. Commercial pure β-glucan gel was firmer than BBG gel at the same concentration level, but it was less firm compared to all the other BBG gels when the concentration of pure β-glucan was taken into account. It might be that impurities present in BBG gum (proteins, pentosans, starch) also play an important role in the structure of gel network by forming additional crosslinks between β-glucan chains and firming the gel structure. In addition, interaction of β-glucan with other food components is not known yet. β-Glucan show potential for use as a gelling agent but conditions for maximum stability need to be optimized.

The main drawback for the wider food applications of β-glucan as a thickener, stabilizer, fat replacer or gelling agent is its high price ($1000/kg). However, this is the current price of stable HV oat β-glucan from a small scale

plant and it is expected to drop with larger scale production in the near future. Barley should also be considered as a starting material for large scale β-glucan production. Utilization of other barley fractions rich in starch and proteins should be evaluated. Advances in technology and a better understanding of the behavior of β-glucan will lead to new applications in a wide range of products. Only then its health benefits can affect the wider population.

Thus, further research focused on the interactions of β-glucan with other food components and resolving of stability problems should prepare the field for better exploitation of such a natural resource.

One approach may be the modification of β-glucan, in which case its rheological properties and health benefits of modified β-glucans require investigation. With joint resources of scientists and industry, the era of β-glucan is yet to come.

5.1. REFERENCES

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22: 163-170.

Cave, N.A., Wood, P.J., and Burrows, V.D. 1990. The nutritive value of naked oats for broiler chicks as affected by dietary additions of oat gum, enzyme, antibiotic, bile salt and fat-soluble vitamins. Can. J. Anim. Sci. 70: 623-633.

Fastwood, M. 1987. Dietary fiber and the risk of cancer. Nutr. Rev. 45: 193.

Gosain, K. 1996. Long Term Effects of Barley Bread Products on Metabolic Control of Non Insulin Dependent Diabetes Mellitus. MSc. Thesis, University of Alberta, Edmonton

Health and Welfare Canada. 1990. *Nutrition Recommendations*. The Report of the Scientific Review Committee.

Parry, R.M. Jr. 1974. Milk coagulation and protein denaturation. Ch. 11 in *Fundamentals of Dairy Chemistry.* B.H. Webb, A.H. Johnson, and J.A. Alford (Eds.) p. 603-661. AVI Publishing Co., Westport, CT.

Ozimek, L. 1996. Personal communication. Department of Agricultural Food and Nutritional Science. University of Alberta, Edmonton, AB.